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THE FUNGICIDAL EFFECT OF VEGETABLE-TANNED LEATHER AND VARIOUS DISINFECTANTS ON *TRICHOPHYTON GYPSEUM* AND *T. INTERDIGITALE*[1]

BY C. O. FULTON[2], N. E. GIBBONS[2], AND R. L. MOORE[3]

Abstract

Vegetable-tanned leather had a fungicidal effect on one strain of *Trichophyton gypseum* and two strains of *T. interdigitale* but not on several strains of common mould contaminants. Chrome-tanned leather, vegetable-tanned leather from an old shoe, and vegetable-tanned leather leached overnight in running water did not possess fungistatic or fungicidal properties.

Of a number of disinfectants tested under conditions similar to those occurring during the fat-liquoring operation, phenylmercuric acetate was the most effective, destroying the above organisms in a concentration of 1 : 100,000. Under the same conditions a 1 : 1000 dilution of commercial formalin was required.

Introduction

The practice of salvaging shoes has raised the question of infection of the shoe repairers and of those who wear the reconditioned shoes. Although other organisms may be involved, those causing 'athlete's foot' are considered to be most important.

Fungous infections of the hands are apparently rare and there is little danger to the repair men. Sulzberger *et al.* (13) indicated that the organisms of athlete's foot are widely distributed and that the disease is not very contagious, inferring that infection depends more on the susceptibility of the host than on exposure to the organisms. On the other hand Legge (9) found that in 1000 college freshmen the percentage of students with athlete's foot increased from 51.5 to 78.6% during the first semester; Berberian (2) has recommended decontaminating shoes of infected individuals with formaldehyde to prevent reinfection; and precautions are being taken in the United States Army against the transmission of the fungus by means of shoes (1). Thus, in the absence of conclusive proof that shoes are not a factor in the spread of the infection, an investigation on methods of disinfecting salvaged shoes seemed advisable.

[1] *Manuscript received June 19, 1944.*
Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as N.R.C. No. 1340.

[2] *Bacteriologist.*

[3] *Laboratory Assistant.*

Experimental Procedures and Results

Before rebuilding, the shoes in question are softened in a 'fat liquor' consisting of an emulsion of various oils in water. The shoes are left in the fat liquor for 15 min. at room temperature and then drained for approximately 18 hr. in a covered tank. It seemed logical to add a fungicide to this fat liquor and most of the work reported here was based on this suggestion.

Effect of Chrome- and Vegetable-Tanned Leather on Trichophyton gypseum

That part of the shoe most likely to be contaminated is the insole, which is usually made of vegetable-tanned leather, and the adjacent upper, which is usually chrome-tanned leather. Therefore prior to testing the disinfectants, tests were made to estimate how long a strain of *Trichophyton gypseum*[1] would remain viable in contact with either chrome-tanned or vegetable-tanned leather that had been fat liquored.

The two types of leather were cut into pieces, 1 in. $\times$ $\frac{1}{2}$ in., and sterilized by holding in an oven at 110° C. for a week. Pieces of each type of leather were dipped into a heavy suspension of *T. gypseum* in fat liquor, drained against the side of the flask, and placed in sterile Petri dishes. At intervals pieces were removed from the dishes, placed in a flask containing 25 ml. of Sabouraud's broth, shaken thoroughly to remove surviving spores and mycelia, and the leather removed. The flasks of broth were incubated for two weeks at room temperature (25° C. $\pm$ 2°). Mould contaminants appeared in some of the flasks, but could readily be distinguished from the typical velvety white surface growth of *T. gypseum*.

The results indicate a striking difference in the survival time on the two types of leather. On chrome-tanned leather the organism was viable at the end of 30 days, but not 40, whereas on vegetable-tanned leather organisms could be detected at intervals up to two hours but not at four hours or thereafter.

The difference in fungistatic action between the leathers is also shown in Fig. 1. Pieces of vegetable- and chrome-tanned leathers were placed on heavily inoculated plates of Sabouraud's agar and incubated at room temperature. Diffusible substances from the vegetable-tanned leather produced a zone of inhibition when the plate was seeded with either *T. gypseum* or the two strains of *T. interdigitale*. No such phenomenon occurred with chrome-tanned leather. Although no tests were made to determine whether the diffusible substances were fungicidal in this test, from the preceding experiment it would appear that the organisms are killed. Mould contaminants such as *Aspergillus fumigatus*, *A. flavus*, *Rhizopus nigricans*, and an unidentified species of *Alternaria* were not inhibited by diffusible substances from vegetable-tanned leather. This is illustrated by Fig. 1, C. The leather is vegetable-tanned and the inhibitory effect on *T. interdigitale* can be seen.

[1] *Kindly supplied by Dr. E. S. Keeping, Edmonton, Alta., who states that this species appears to be one of the three most common fungi causing ringworm in North America (5). Strains 4807 and 4808 of T. interdigitale were obtained from the American Type Culture Collection.*

PLATE I

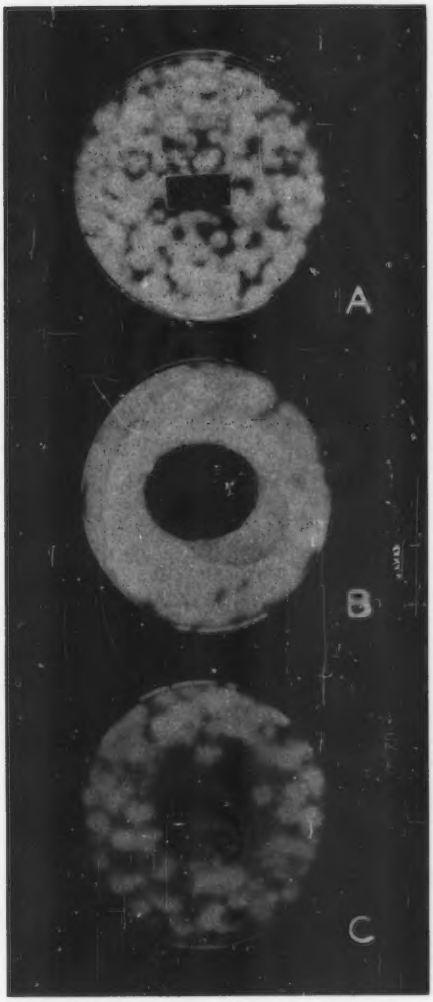

FIG. 1. *A—Absence of inhibition with chrome-tanned leather on plate seeded with T. interdigitale.*

B—Inhibition of Trichophyton interdigitale by vegetable-tanned leather.

C—Rhizopus nigricans growing on and around vegetable-tanned leather and over zone inhibitory to T. interdigitale.

However, *Rhizopus nigricans* is thriving on and around the leather. This seems to indicate that the fungistatic compounds in the leather are specific for at least the species of *Trichophyton* tested but not for many other fungi.

To determine whether the inhibitory substances in the leather were the unfixed tannins or the glucose and magnesium sulphate commonly used for weighting, commercial samples of insole leather were obtained after removal from the tanning vats. One sample was dried without further treatment. The other was thoroughly leached and then 'weighted' with glucose and magnesium sulphate as in commercial practice. The sample that was not leached and not weighted produced a large zone of inhibition similar to that illustrated in Fig. 1, B. The sample that was leached and then weighted with glucose and magnesium sulphate produced a very slight zone of inhibition. Thus it would appear that the soluble tannins and not the glucose and magnesium sulphate are responsible for the phenomenon. Three of the compounds usually found in vegetable-tanning liquors, tannic acid, gallic acid, and pyrogallic acid, were inhibitory (Table I). It is possible that the fungistatic and fungicidal action of the vegetable-tanned leather was due to the combined action of these and similar water soluble compounds. This was supported by the fact that the inhibitory action could not be demonstrated in leather that has been leached overnight in running water. Samples from the insole of an old shoe were also not inhibitory and it is probable that most

TABLE I

FUNGISTATIC ACTION OF VARIOUS DISINFECTANTS IN SABOURAUD'S AGAR AGAINST *Trichophyton gypseum* AND *T. interdigitale* (INCUBATED ONE MONTH AT ROOM TEMPERATURE 21° C.)

Disinfectants	Concentrations of disinfectant, %					
	1	0.1	0.01	0.001	0.0001	0.00001
Phenylmercuric acetate	—	—	0	0	0	+
Merthiolate	—	—	0	0	0	+
'Dihexylin'*	0	0	0	0	+	+
Sodium pentachlorophenate	0	0	0	0	+	—
Thymol	0	0	0	+	+	+
Pyrogallic acid	0	0	0	+	+	+
Commercial formalin	0	0	0	+	+	+
Commercial 'Izal'	0	0	+	+	+	+
Commercial 'Zephiran'**	0	0	+	+	+	+
'Phemerol'*	0	0	+	+	+	+
Mercuric chloride	—	0	+	+	+	+
Tannic acid	0	0	+	+	+	+
Betanaphthol	0	0	+	+	+	+
Copper sulphate	0	+	+	+	+	+
Gallic acid	0	+	+	+	+	+
'Dettol'	+	+	+	+	+	+
Sodium thiosulphate	+	+	+	+	+	+

\+ = *Growth.*
0 = *No growth.*
* *Supplied by Parke Davis Co.*
** *Supplied by Alba Pharmaceutical Co., now Winthrop Chemical Company.*

of the water soluble fractions had been leached out while the shoe was being worn. Although new vegetable-tanned leather is fungicidal against these organisms it is probable that this property would soon be lost, just how soon depending on how rapidly the water soluble fractions were leached out.

Effect of Disinfectants in Fat Liquor on T. gypseum and T. interdigitale

As a preliminary test, the fungistatic action of 17 disinfectants in Sabouraud's agar was determined. The results are summarized in Table I and apply to all three strains of organism studied. The concentrations given are the dilutions made of the pure or commercial compounds as received.

Eleven of the most active fungistatic agents were then tested for their ability to kill the organisms in fat liquor under conditions approximating those obtained in the fat liquoring operation. Chrome-tanned leather was used because of the inhibitory action of vegetable-tanned leather noted above. *T. gypseum* was used in this test because *T. interdigitale* sometimes failed to survive the 18 hr. draining period even when no disinfectant had been added. Fat liquor A* was used in these tests. Fat liquor B**, which was not obtained until later in the work, was tested with the best of the disinfectants with essentially the same results.

The organism was grown in flasks containing coarse sand and 500 ml. of Sabouraud's broth until a heavy matted growth appeared on the surface. The broth was then decanted off leaving the matted growth and sand in the flask. To this was added 500 ml. of sterile fat liquor and the flask shaken vigorously to produce a heavy suspension of the organism in the liquor. This heavily contaminated fat liquor served as a diluent for making tenfold dilutions of the disinfectants. A sterile piece of chrome-tanned leather (1 in. × ½ in.) was dropped into each dilution and after 15 min. was placed in a Petri dish and held at room temperature 18 hr. to correspond to the draining period. Each piece of leather was then dropped into a flask containing 100 ml. of Sabouraud's broth. Tests showed that a piece of leather of the size used absorbed an average of 0.4 ml. of fat liquor. It is apparent from Table I that the disinfectant carried by the test piece was diluted beyond the fungistatic limit in 100 ml. of broth. If no growth appeared after 15 days' incubation at room temperature it was concluded that that concentration of disinfectant in fat liquor was fungicidal. The results are given in Table II.

Phenylmercuric acetate was effective in killing *T. gypseum* in a concentration of 1 : 100,000 when allowed to act 18 hr. A 1 : 1000 dilution of formalin or dihexylin also kills this organism in the same time. If a 15 min. exposure only is used, as might be done in washing the shoes before fat liquoring, a concentration of 1 : 10,000 phenylmercuric acetate or 1 : 100 formalin is necessary.

Since phenylmercuric acetate was one of the cheapest of those compounds effective in low concentration, patch tests were performed to ascertain its

* *Leather Softener, supplied by North American Leather Co.*
** *Lexol, supplied by A. G. Baker & Co., Toronto.*

TABLE II

FUNGICIDAL ACTION OF VARIOUS DISINFECTANTS AGAINST *Trichophyton gypseum* IN FAT LIQUOR UNDER CONDITIONS SIMILAR TO THOSE OCCURRING DURING THE FAT LIQUORING OPERATION

Disinfectant	Concentrations of disinfectant, %					
	5	1	0.1	0.01	0.001	0.0001
Phenylmercuric acetate	—	—	—	0	0	+
Commercial formalin	—	0	0	+	+	+
'Dihexylin'	—	0	0	+	+	+
Pyrogallic acid	—	0	+	+	+	+
Pentachlorophenate	—	0	+	+	+	+
Thymol	—	0	+	+	+	+
Mercuric chloride	—	0	+	+	+	+
Commercial 'Zephiran'	—	+	+	+	+	+
'Izal'	0	+	+	+	+	+
Copper sulphate	—	+	+	+	+	+
'Dettol'	—	+	+	+	+	+

0 = *Absence of growth after four weeks' incubation in 100 ml. Sabouraud's broth at 25° C.*
\+ = *Growth under similar conditions.*

effect on the skin. Pieces of chrome leather 1 in. × 1 in. were soaked 15 min. in fat liquor containing 1 part of phenylmercuric acetate to 100,000 parts of fat liquor. The leather was allowed to drain and then tested on a group of six men and two women, one of whom had a history of a dermatitis caused by mercuric chloride. The skin beneath the 1-in. square patch of leather was examined at 24 hr. intervals for three days, but no reaction of any kind was observed.

Discussion

The observation that vegetable-tanned leather has a fungicidal action on three strains of *Trichophyton* may possibly account for the low percentage of successful isolations Jamieson (8) made from the insoles of supposedly contaminated shoes. Doelger (4) has attributed the relatively small number of moulds in tan liquors to the inhibitory action of the tannins. However, in this work although strains of *Trichophyton* were inhibited by vegetable-tanned leather other moulds were not and it is probable that the concentration of tannins remaining in the leather were sufficient to prevent the growth of *Trichophyton* species but not enough to inhibit many other types of fungi.

The striking fungistatic and fungicidal action of phenylmercuric acetate noted in this work corresponds to that observed for other phenylmercuric compounds by others (6, 7, 11, 14). Shuttleworth (12) used phenylmercuric acetate in a dilution of 1 : 200,000 to prevent mould growth in tan liquors.

In the concentrations used very little disinfectant would be carried in the leather. Based on the amount of fat liquor absorbed by new leather, a square foot of leather would contain 1 ml. of phenylmercuric acetate if soaked in a 1 : 100,000 dilution. In view of the concentrations used by others (3, 9, 10, 12) it is not likely that these concentrations would have any effect on the skin of the feet.

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DRIED WHOLE EGG POWDER

XV. THE GROWTH OF *SALMONELLA* AND OTHER ORGANISMS IN LIQUID AND RECONSTITUTED EGG[1]

By N. E. Gibbons[2], R. L. Moore[3], and C. O. Fulton[2]

Abstract

Curves are presented showing the growth in liquid egg of *Salmonella bareilly, S. manhattan, S. typhi-murium, S. oranienburg, S. typhi, Escherichia coli, Aerobacter aerogenes, Staphylococcus aureus, Streptococcus fecalis* and *S. pyogenes*, and of *Salmonella bareilly* in reconstituted egg. *Streptococcus pyogenes* does not grow in egg and dies off rapidly at temperatures above 60° F. (15.6° C.). The other organisms generally grow well in liquid egg at temperatures above 60° F. (15.6° C.). Liquid and reconstituted egg should therefore be maintained well below this temperature to prevent the multiplication of *Salmonella* and other possible pathogens.

Introduction

Organisms of the *Salmonella* group have been reported in dried egg powder (1, 2, 6) but the number is usually small (2). Although drying kills most of the organisms, the greater the number of organisms in the liquid egg the greater the number likely to survive the drying process (3). Since these organisms may multiply in the reconstituted egg and form a health hazard, it was of interest to determine at what temperatures growth may occur in liquid and reconstituted egg. *Staphylococcus aureus* and *Streptococcus pyogenes*, which are also of public health significance, as well as other organisms commonly present on eggs, were included in the study.

Methods

To simplify the task of making counts, sterile egg melange was used. Eggs, one day old, were used for most of the work, although commercial Grade A eggs were found to yield a high percentage of sterile meats. The method used to obtain sterile meats was essentially that of Rosser (4). One egg is allowed to soak in a 500 p.p.m. chlorine solution while the previously treated egg is being opened. The egg is held between two small ring clamps fastened to a bar, bent at right angles, which is in turn fastened to a ring stand so that the egg may be inverted by loosening the clamp at the ring stand (Fig. 1, A). The large end of the egg is painted with tincture of iodine and a triangular piece of shell cut out with a sterile abrasive wheel in an electric hand drill. The shell and outer membranes are removed with sterile forceps. The egg is then inverted, the small end painted with iodine, and a small hole made in it with the drill. The inner membrane at each end is pierced with a platinum

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[2] *Bacteriologist, Food Investigations.*

[3] *Laboratory Assistant, Food Investigations.*

wire and the egg contents blown into a sterile wide mouthed flask by means of compressed air filtered through a Seitz filter (Fig. 1, B). The flasks, each containing one egg, are incubated overnight and checked the next day for growth by subculturing. The sterile eggs are then poured into a large Seitz filter fitted with a fine wire screen in place of the filter disk, and the egg is forced through the screen by compressed air (Fig. 1, C). The egg is caught in a delivery bottle, mixed, dispensed into sterile flasks as needed and again checked for sterility. Using this method sterile meats were obtained from 80 to 95% of Grade A commercial eggs.

Most of the organisms used in this study were isolated from egg powder: *Salmonella bareilly; S. manhattan; S. oranienburg; S. typhi-murium; Escherichia coli; Aerobacter aerogenes;* and *Streptococcus fecalis*. The other organisms used were: *Salmonella typhi*, Hopkins disinfectant strain; *Staphylococcus aureus*, Wood 46, a non-enterotoxin producing strain; and four strains of *Streptococcus pyogenes*, Group A* (Matthews Type 25 and three strains isolated from scarlet fever patients, Types 1, 4, 12-14).

Growth was followed by the procedure outlined below. The melange was inoculated with 24-hr. broth cultures of the organisms, distributed into the necessary number of flasks and incubated. The liquid egg was heated rapidly to the desired temperature in a water-bath before being placed in the incubator. In the first experiments the temperatures used were 98.6°, 86°, 75°, 68°, 60°, 52°, and 45° F. (37°, 30°, 24°, 20°, 15.6°, 10.6°, and 7.2° C.); in later experiments 90°, 80°, 70°, 60°, 50°, and 40° F. (32.2°, 26.7°, 21.1°, 15.6°, 10.0°, and 4.4° C.). Aliquots were removed at 6, 12, and 24 hr. at the higher temperatures and also at 48 hr. at the lower temperatures since under practical conditions liquid or reconstituted egg would not likely be held for longer periods. Appropriate dilutions were plated on proteose-peptone tryptone agar. The streptococci were plated on heart infusion agar. All plates were incubated at 98.6° F. and counted after three days.

Since it is practically impossible to obtain sterile spray dried egg powder, growth curves in reconstituted egg are difficult to obtain. Curves for *Salmonella bareilly* were obtained by using powder free of other sulphide-producing organisms and following growth by the most probable number technique previously described (2).

Results

The growth curves of *Salmonella* in liquid egg are shown in Fig. 2 (A and B) and Fig. 3 (A, C, and D). In each there is a decided break in the rate of growth around 60° F. (15.6° C.). Above this the rate increases with increasing temperature to about 86° F. Below 60° F. there is little or no increase and in some instances a decrease. Only one strain (*S. manhattan*, Fig. 2, B) showed rapid growth at 60° F.: in all others there was a definite lag of at least 12 hr. followed by a fairly rapid increase. For practical purposes it is evident that if the egg liquid is kept below 60° F. there is little

* *Kindly supplied by Dr. E. T. Bynoe, Laboratory of Hygiene, Department of Pensions and National Health, Ottawa.*

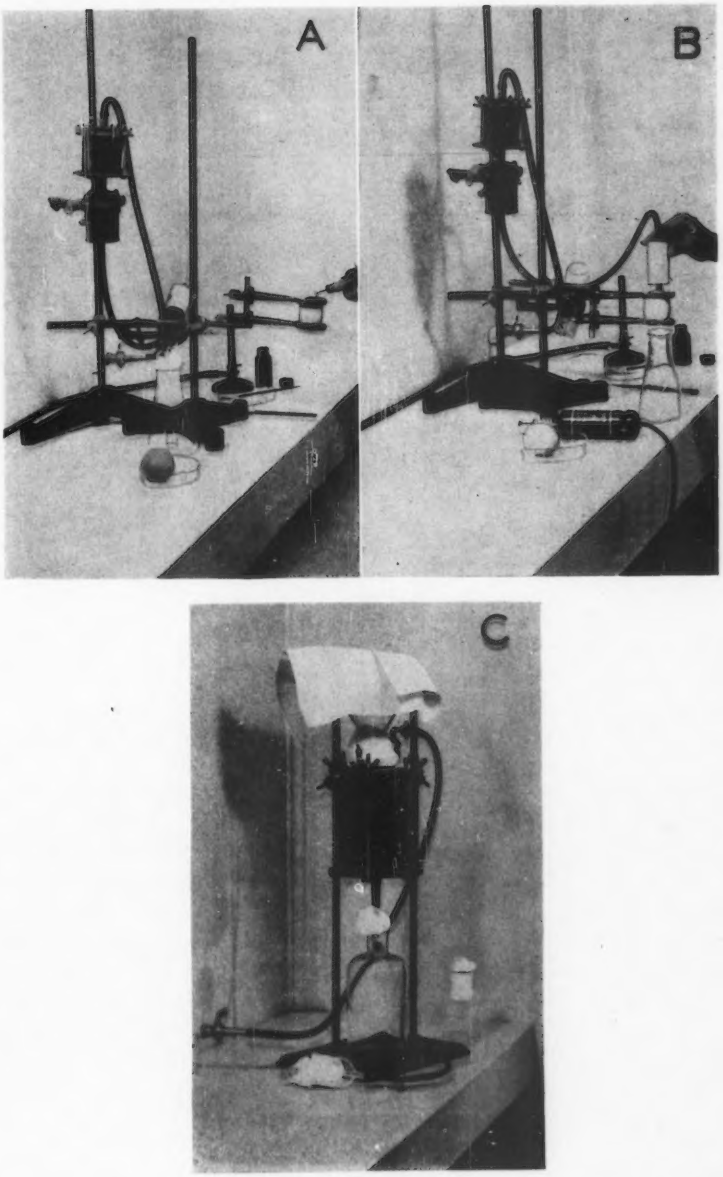

FIG. 1. *Apparatus for obtaining sterile egg meats. A. Cutting the shell. B. Blowing out the contents. C. Seitz filter and apparatus for straining and dispensing liquid egg.*

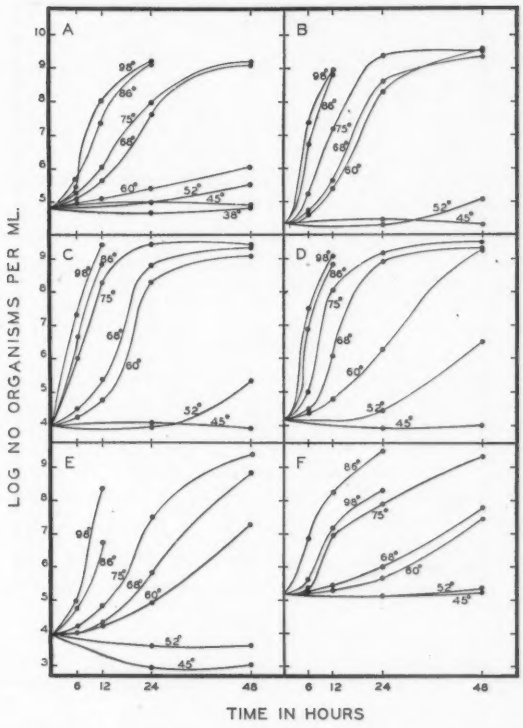

FIG. 2. *Growth curves in liquid egg at 98°, 86°, 75°, 68°, 60°, 52°, and 45° F. of: A,—Salmonella bareilly; B,—S. manhattan; C,—E. coli; D,—A. aerogenes; E,—S. typhi; and F,—Staphylococcus aureus.*

danger of multiplication of *Salmonella* organisms over the period studied. At room temperature (68° F.) there is a fairly rapid growth and the final number is at least as great in 48 hr. as at the higher temperatures. This agrees with work recently reported for canned foods (5).

Salmonella bareilly does not appear to grow quite as well in reconstituted egg (Fig. 3, B) as in liquid egg (Fig. 3, A). This difference may be due in part to the different method of estimating the number of organisms. Although there is a definite lag period the original decreases at 60° F. and 70° F. are possibly fortuitous.

The growth of *E. coli* and *A. aerogenes* (Fig. 2, C and D) is essentially the same at the higher temperatures. The strain of *A. aerogenes* used is not affected by the lower temperatures in the same way as *E. coli*. Below 60° F. *Salmonella typhi* (Fig. 2, E) decreased in numbers over the period studied.

Staphylococcus aureus (Fig. 2, F) was the only organism used that grew more rapidly at 86° than at 98° F. It, however, does not grow as well at room temperatures as the *Salmonella* strains.

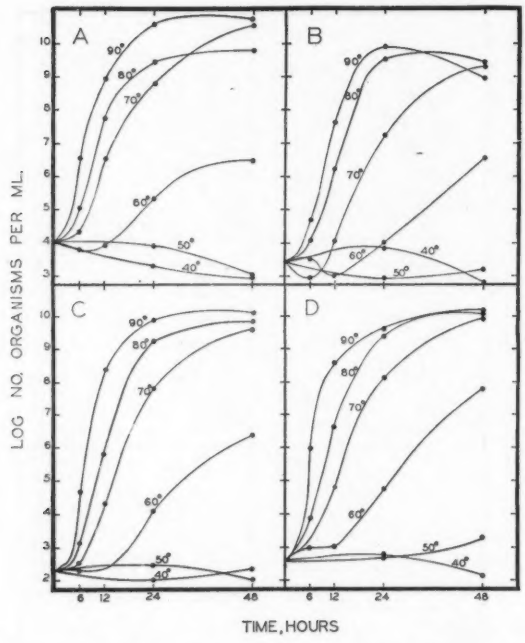

FIG. 3. *Growth curves of Salmonella bareilly in: A,—egg liquid; and B,—reconstituted egg powder; and of C,—S. typhi-murium and D,—S. oranienburg, in liquid egg. Temperatures in ° F.*

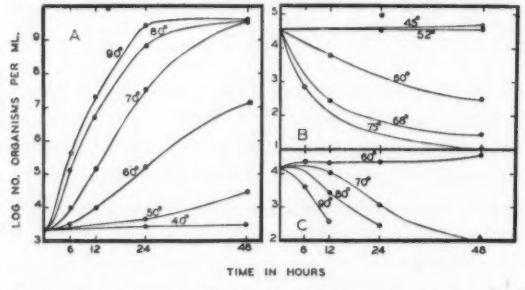

FIG. 4. *Growth curves in liquid egg of: A,—Streptococcus fecalis; B,—S. pyogenes (Matthews strain); and C,—S pyogenes, Group A. Temperatures in ° F.*

Streptococcus fecalis does not grow as rapidly at the higher temperatures as the majority of other organisms (Fig. 4, A). None of the strains of *Streptococcus pyogenes* grew in liquid egg. They survive at the lower temperatures but die off rapidly above 60° F. The curves obtained for two strains are presented (Fig. 4, B and C). Similar curves were obtained for the other two strains.

Summary

Group A streptococci do not grow in liquid egg. This is probably one explanation of the fact that they are not usually found in egg powder*.

For the other organisms studied, 60° F. seems to be the dividing line between rapid growth and little or no growth over a 48 hr. period. At this temperature, there is usually a lag period of about 12 hr., which may or may not be followed by rapid growth. In actual drying practice, where liquid egg is seldom held longer than 12 hr. and usually at temperatures of 45° F., there would be little danger of multiplication. However, if reconstituted egg were allowed to stand overnight at room temperature multiplication would take place.

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* *Unpublished data.*

SOME PHYSICAL PROPERTIES OF A SAMPLE OF ALBERTA BITUMINOUS SAND[1]

By K. A. Clark[2]

Abstract

Some physical properties of a sample of bituminous sand from the Abasand Oils Ltd. quarry have been determined. The sample contained about 17% bitumen and 1% water. The porosity of the sample in its natural state of packing was 39 to 40%, while saturation of the pore space with bitumen was 80 to 85% and with water, 3.5 to 4.5%. The coefficient of thermal conductivity of the sample in its natural state of packing was 0.0035 in c.g.s. units and at 45°C. The specific heat of the bitumen content of the sample was 0.35 and of the mineral content, 0.18 for the temperature range 0° to 100° C. The bitumen had a calorific value of 17,860 B.t.u. per lb.

The measurements of physical properties reported in this paper were made on material from a single lump of bituminous sand from the Abasand Oils Ltd. quarry in Horse River valley, near Fort McMurray, Alberta. The lump was a large one and consisted of bituminous sand in the natural state of packing that existed in the bed from which it came. It was shipped to the laboratory at the University of Alberta during freezing weather and it remained frozen until the time at which the measurements were made. The nature of the bituminous sand is revealed by the results of the analyses recorded in Table I

TABLE I

Bitumen, water, and mineral matter contents of samples from a large lump of bituminous sand from the Abasand Oils Ltd. quarry

Sample	Percentage of		
	Bitumen	Water	Mineral matter
Outside of lump	17.0	0.7	82.3
Outside of lump	17.0	0.7	82.3
Inside of lump	17.1	0.9	82.0
Inside of lump	17.1	0.9	82.0

Sieve analyses of mineral matter in bituminous sand samples

Sample	Per cent retained on mesh				Passing 200 mesh
	50	80	100	200	
Outside of lump	0.1	3.4	25.1	95.6	4.4
Inside of lump	0.1	3.4	25.6	96.8	3.2

[1] *Manuscript received June 20, 1944.*
Contribution from the Research Council of Alberta, University of Alberta, Edmonton, Alta.
[2] *Research Engineer.*

Efforts to attain a high degree of accuracy in measurements of physical properties of bituminous sand are not warranted. The material varies quite widely in all aspects of composition from point to point in the bituminous deposit (1). Intelligent use of the values for the physical properties of the particular bituminous sand sample under consideration can be made if the fact of variability is appreciated.

Specific Gravity, Porosity, and Liquid Saturation

The amount of pore space in the mineral aggregate of bituminous sand as it lies in the beds of the deposit and the degree to which such pore space is filled with oil and water are matters of interest. Porosities and saturations undoubtedly vary greatly. Measurements on one well impregnated sand gives some hint of the order of magnitude of these values.

The weight of bituminous sand in its natural state of packing is a practical question. The specific gravities of undisturbed lumps are determined incidental to porosity and saturation measurements.

The large lump of bituminous sand from the Abasand quarry, weighing about 200 lb., was cut into 4- or 5-in. cubes. This was done out-of-doors in winter weather by means of an electrically heated nichrome wire. During the process, some irregular shaped chunks of bituminous sand broke away. These were used for the porosity and saturation measurements. All material was kept in outdoor storage.

The key measurement for porosity determinations is the determination of the volume of a lump of the material in its undisturbed state of packing. In the case of the bituminous sand, this was done by immersing the lump of sand in mercury and by weighing the displaced mercury (3). A glass dish of suitable size and with a ground top was more than filled with mercury. A glass plate carrying three metal prongs on its under side was pressed on to the ground top of the dish, thus squeezing out excess mercury. The lump of bituminous sand was then floated on the mercury in the dish and forced beneath the surface with the metal prongs of the glass plate. Mercury equal in volume to that of the lump was forced out of the dish during the process. The mercury was weighed and its volume calculated, using mercury density data.

The specific gravity of the mineral aggregate of the bituminous sand was determined and found to be 2.65. The specific gravity of the oil in the bituminous sand was also determined and found to be 1.017 at 25° C./25° C.

The porosities and oil saturations shown in Table II were calculated using the following formulae:

$$\frac{100\ (\text{Volume of lump} - \text{volume of sand grains})}{\text{Volume of lump}} = \%\ \text{Porosity}$$

$$\frac{100\ (\text{Volume of oil in lump})}{\text{Volume of lump} - \text{volume of sand grains}} = \%\ \text{Oil saturation}$$

TABLE II

SPECIFIC GRAVITIES, POROSITIES, AND SATURATIONS OF LUMPS OF BITUMINOUS SAND IN THEIR NATURAL STATE OF PACKING

Lump No.	Weight, gm.	Volume, cc.	Specific gravity	Porosity, %	Per cent saturation		
					Oil	Water	Total
Inside							
1	46.5	23.4	1.99	38.5	87.0	4.7	91.7
2	45.2	22.7	1.99	38.3	87.5	4.7	92.2
3	26.4	13.5	1.96	39.5	83.5	4.5	87.7
4	29.7	15.1	1.97	39.1	84.6	4.6	89.2
5	44.4	22.5	1.97	38.9	85.3	4.6	89.9
6	33.5	17.2	1.95	39.7	82.6	4.4	87.0
7	48.9	24.5	1.99	38.1	88.1	4.7	92.8
8	35.2	18.3	1.92	40.4	80.0	4.3	84.3
9	47.7	24.2	1.97	39.0	85.2	4.6	89.8
10	33.0	17.2	1.92	40.6	79.5	4.3	83.8
Av.	—	—	1.96	39.2	84.3	4.5	88.8
Outside							
11	18.3	9.58	1.91	40.7	78.3	3.3	81.6
12	27.6	14.25	1.94	39.9	81.4	3.3	84.7
13	46.3	23.8	1.95	39.6	82.2	3.4	85.6
Av.	—	—	1.93	40.1	80.6	3.3	83.9

The spread in values for specific gravities, porosities, and saturations in Table II are probably due both to experimental error and to actual variations of the physical properties.

The water content of the bituminous sand lump used was abnormally low. For material from the Abasand quarry the normal water content is from 2 to 3% as the oil content varies from 17 to 15%. It is interesting to note that if the material examined had had a water content of 3%, the total saturation with oil and water would have been almost complete. Supersaturation of bituminous sand occurs sometimes at the face of exposures where there is no lateral support. Where this happens the sand shakes like a jelly when pressed. It may even flow and cause a bituminous sand slide.

Heat Conductivity of Bituminous Sand

The thermal conductivity of bituminous sand enters into various practical problems. For instance, various workers have tried to win the oil from the sands, *in situ*, by heating the sand beds from bore holes. Some time spent on determining the heat transfer through bituminous sand would have saved still more time and much money that was spent on hopeless experimentation. Again, the rate of penetration of cold into bituminous sand has a bearing on the practical problem of mining in winter time. Other problems regarding plant design that involve the thermal conductivity of bituminous sand will arise.

Two 5-in. cubes of bituminous sand cut from the large lump without disturbing the natural packing were used for the determination of the coefficient of thermal conductivity. The blocks were set one on top of the other, with faces that fitted together closely in contact. A straight piece of resistance wire was shoved vertically through the centre of the top block and right on down through the lower block. Iron-constantan thermocouples were placed on the horizontal plane, between the two blocks, at measured distances away from the electric resistance wire. A state of steady flow of heat through the bituminous sand block was established by passing a constant current through the resistance wire until the readings of the two thermocouples showed no change with time. With steady conditions established, the flow of heat was in accordance with the following equation (2):

$$\frac{I^2R}{4.19} = \frac{2\pi\ k\ (\theta_1-\theta_2)}{2.303\ \log_{10}\frac{r_2}{r_1}}$$

where I = amperes of current flowing in the resistance wire through the blocks of bituminous sand,

R = resistance in ohms of 1 cm. length of the wire,

$\frac{I^2R}{4.19}$ = calories of heat entering and leaving each horizontal slice 1 cm. thick of the blocks of bituminous sand each second,

θ_1 = temperature in ° C. of a cylindrical surface of the bituminous sand at distance r_1 centimetres from the axial resistance wire,

θ_2 = temperature in ° C. of a cylindrical surface of the bituminous sand at distance r_2 centimetres from the axial resistance wire,

k = coefficient of thermal conductivity in c.g.s. units.

The equation given applies, strictly, to a cylinder of bituminous sand with a resistance wire along its axis. Consequently the blocks of sand should have been trimmed into cylinders. Doing so probably would have introduced more errors than it would have eliminated. However, measurements were made on bituminous sand repacked into cylindrical shape in a split concrete mould 3 in. in diameter and 6 in. long. Two half-cylinders were moulded. Along the face of one of these the resistance wire and the thermocouples were fastened. On clamping the two halves of the mould together again, a cylinder of bituminous sand was obtained with the various wires in place. By this means, the attempt was made to follow the change in the coefficient of thermal conductivity as the oil content of the bituminous sand was reduced by admixture of oil-free mineral aggregate. The attempt was not very successful. The thermal conductivity of repacked material is affected too much by the degree of packing attained. However, the results obtained are shown in Table III.

The heat conductivity measurements indicate that the coefficient of thermal conductivity of bituminous sand of 17% oil content in its natural state of packing is about 0.0035 in c.g.s. units at 45° C., or thereabouts; that repacked

TABLE III

RESULTS OF THERMAL CONDUCTIVITY MEASUREMENTS ON UNDISTURBED AND REMOULDED BITUMINOUS SAND

Electrical resistance of axial wire, 0.0081 ohm/cm.

Bituminous sand sample	I, amp.	r_1, cm.	r_2, cm.	θ_1, ° C.	θ_2, ° C.	k
Bituminous sand in natural state of packing; oil content, 17%	6.8	0.65	2.6	47.6	42.0	0.0035
"	6.9	0.85	3.2	47.8	42.2	0.0035
Remoulded bituminous sand; oil content, 17%	8.4	1.2	3.0	47.8	40.4	0.0027
"	7.0	0.6	3.0	47.8	40.3	0.0032
"	7.0	0.65	2.25	48.8	42.7	0.0031
"	7.0	1.15	2.15	46.4	43.3	0.0030
Remoulded bituminous sand; oil content, 11.7%	6.8	0.7	2.4	50.6	42.0	0.0021
Remoulded bituminous sand; oil content, 8.6%	7.0	1.3	3.0	36.8	31.6	0.0024
Remoulded bituminous sand; oil content, 3%	6.6	0.9	3.0	42.4	32.6	0.0017

bituminous sand has a somewhat lower coefficient than sand in its natural state of packing; and that the coefficient decreases as the oil content decreases.

An approximate analysis of the flow of heat in two practical bituminous sand situations was attempted, using the coefficient of thermal conductivity of 0.0035. It was deduced that if a temperature of 2000° F. were maintained for 24 hr. in a combustion chamber at the bottom of a bore hole into the bituminous sands, the temperature at 24 in. distance from the chamber would be 212° F.; and that at the end of 72 hr. the temperature of 212° F. would be 43 in. distant. This calculation is consistent with an actual experiment made by an *in situ* recovery enthusiast. The combustion chamber was about ten feet from the face of an exposure and was dug into after the experiment. It was deduced, also, that if the weather remained steadily at −20° F. for 24 hr., the temperature would be at the freezing point at a depth of $6\frac{1}{2}$ in. into the bituminous sand; and that after one month of such weather, the freezing point temperature would be at a depth of 36 in. It was assumed for the calculations that the normal temperature of the bituminous sand beds was 40° F.

Specific Heats of Bituminous Sand and of Its Constituents

The specific heats of the bituminous sand from the Abasand quarry and of its mineral matter and oil constituents were determined by simple measure-

ments in a thermos bottle. The heat required to raise the inside temperature of the bottle one degree centigrade was measured by observing the inside temperature, introducing a known weight of heated water, shaking, and again observing the inside temperature. Measurements were then made on the mineral aggregate of the bituminous sand, and on the bituminous sand itself. The specific heat of the oil in the sand was deduced by calculation. However, one direct measurement on the oil was made. Results are shown in Table IV.

TABLE IV

SPECIFIC HEATS OF A BITUMINOUS SAND AND OF ITS MINERAL MATTER AND OIL CONSTITUENTS IN THE RANGE 0–100° C.

Material	Specific heat
Bituminous sand	0.218
Oil, 17.1%	0.214
Water, 0.9%	0.222
Average	0.218
Mineral matter	0.181
	0.170
	0.181
	0.194
	0.181
	0.170
Average	0.180
Oil (calculated)	0.350
(measured)	0.351

For purposes of calculation of heat balances and for other plant problems, a specific heat of 0.18 cal. per gm. per degree centigrade for the mineral matter and of 0.35 for the oil in bituminous sand may be used for temperatures between 0° and 100° C.

Calorific Value and Ultimate Analysis of the Oil Constituent of Abasand Bituminous Sand

The following determinations of the calorific value and of the ultimate analysis of the oil from the Abasand bituminous sand were made in the Fuels Laboratory of the Research Council of Alberta. The oil was extracted from the bituminous sand with benzene, the bulk of the benzene was distilled off, and the rest of it was evaporated by heating for several hours over a water-bath.

TABLE V

CALORIFIC VALUE AND ULTIMATE ANALYSIS OF THE OIL CONSTITUENT OF BITUMINOUS SAND FROM THE ABASAND QUARRY

B.t.u./lb.	17,860	% Nitrogen	0.46
% Carbon	83.00	% Sulphur	3.78
% Hydrogen	10.22	C/H ratio	8.1

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THE SPONTANEOUS HEATING OF FLAXSEED AND SUNFLOWER SEED STORED UNDER ADIABATIC CONDITIONS[1]

By H. R. SALLANS[2], G. D. SINCLAIR[3], AND R. K. LARMOUR[4]

Abstract

The effects of moisture content on the heating of flaxseed and sunflower seed were studied in an adiabatic thermostat. Under these conditions, storage periods of less than two months produced heating in flax at 11.4% moisture and in sunflower seed at 10.5% moisture. Thus it appears that the commercial limits of 10.5 and 9.5% moisture respectively are not too low for these grains.

Evidence is presented to show that an acceleration in the over-all respiration rate of flaxseed and sunflower seed precedes heating. This indicates that heating is caused by active growth of the microflora on the grain and, at moisture levels in the order of those required for safe storage, normal embryonic activity is insufficient to cause heating.

It is suggested that when the relative humidity of the interstitial air in bulk grain exceeds a value of 74% the microflora will grow and heating may ensue. From this it follows that the moisture content of any grain, in equilibrium with air at a relative humidity of 74%, will closely approximate the upper limit permissible for admission to 'straight' grades.

Under certain conditions of moisture and storage grains undergo spontaneous heating. The damage that results materially reduces the value of the grain, and in advanced stages renders it worthless: this phenomenon is known to commercial grain handlers as 'bin burning.' From long experience with wheat, barley, oats, flax, and other grains, practical limits for moisture content have been established; and, if the moisture level in these grains is below certain set values, they can be stored for long periods of time without damage from spontaneous heating. With the introduction of a new crop such as sunflower seed, it is necessary to set a tentative moisture level for safe storage from the results of laboratory studies.

From a study of the respiration of sunflower seed at different moisture levels, Larmour, Sallans, and Craig (4) came to the conclusion that the moisture level for safe storage should be set at 9.5%. This value was based on the assumption that if grain shows an appreciable increase in respiration, as measured by carbon dioxide production, heat must be produced; and, if the grain were stored in bulk, this heat would not be dissipated readily with the result that a temperature rise would occur. Using their technique no appreciable increase in the rate of carbon dioxide production with time could be detected for sunflower seed at a moisture level of 11%. This technique when applied to wheat (3) and flaxseed (4) yielded values of 16 and 12%,

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[2] *Biochemist, Oil Seeds Laboratory.*

[3] *Laboratory Assistant, Oil Seeds Laboratory.*

[4] *Professor of Chemistry, University of Saskatchewan.*

while the established commercial values are 14.5 and 10.5% respectively. Assuming sunflower seed values to be analogous, a correction of minus 1.5% was made on the moisture level of seed that failed to show an acceleration in respiration, and a value of 9.5% was obtained for the upper limit of moisture allowable in 'straight grade' sunflower seed.

As these values obtained from respiration studies are based on an indirect method and an empirical correction factor, it appeared desirable to check them against a direct measure of heating. Since it was not feasible to do this on large bulk storage bins, an adiabatic apparatus was constructed to minimize radiation losses from small laboratory samples.

Apparatus and Materials

Apparatus

An adiabatic thermostat similar in design and principle to that developed by Working and described and illustrated by Ramstad and Geddes (6) was constructed. In their investigations on soybeans these workers determined carbon dioxide production and heating simultaneously. However, their data show no consistent relations between temperature rise and carbon dioxide production. This is not surprising since it is well-known that under conditions of oxygen starvation fungi and bacteria produce other products besides carbon dioxide, therefore, no 'a priori' reason exists for a regular relationship between heating and respiration rates as measured by carbon dioxide production. Hence, for the purpose of the present study the apparatus was simplified by modifying the air feed to the sample and no attempt was made to measure respiration. Laboratory air was drawn through the samples by suction developed by means of an aspirating bottle and an adjustable siphon. To guard against microbiological infection from the air a large drying tube packed with sterile cotton was placed in the air inlet.

It was impossible to obtain the electrical controls specified by Ramstad and Geddes and the following items were substituted, a Leeds Northrup galvanometer (sensitivity 19μv. per mm., period five seconds, resistance 17 ohms), a standard six-volt galvanometer lamp, and a demonstration photoelectric relay. In wiring the heating circuit, three 25-watt bulbs were used as fixed heaters and a 40-watt bulb was used in the control circuit. The temperatures maintained by the fixed heaters were determined and, since the controlled heater had a greater capacity than a unit fixed heater, it was possible to obtain a regular temperature rise when it became necessary to switch in fixed heaters.

The unit was set up in a sub-basement room on a bench rigidly mounted on a concrete wall. This provided an ideal location since the temperature of the room remained virtually constant, and as it was used solely for these experiments there was no vibration or change in light intensity to interfere with the efficient operation of the control circuit.

In making a determination the sample was tempered to the desired moisture level and allowed to stand for 36 to 48 hr. in a closed container adjacent to

the thermostat. It was then transferred to the thermos bottle in the thermostat, the air flow adjusted to 500 ml. per 24 hr., and, after equilibrium had been established, temperature readings were recorded at intervals. As soon as an increase in temperature was noted the rate of air flow was gradually increased to a maximum rate of 2½ to 3 litres per 24 hr.

It is obvious that if the control circuit is improperly adjusted, heating may result from the thermostat operating as a heater. To determine the proper setting of the control, the thermos bottle was filled with water at 40° C. and the control adjusted so that a slight drop in temperature, less than 0.1° C. per 24 hr., occurred. Under these conditions a differential of approximately 0.02° C. was maintained between the inside of the thermos flask and the surrounding air in the thermostat. As an additional check on the control setting, samples were placed in the thermostat slightly above room temperature. It was found in these cases that the temperature of the thermostat would fall till room temperature was reached and after a short induction period a rise in temperature would follow. If this rise were due to heat input from the apparatus, the rate of temperature rise should be relatively constant and a plot of temperature against time should give a linear relation. Reference to the data in Figs. 1, 2, 3, and 4 will show that after the induction period was passed the rate of heating showed an acceleration with time. This may be regarded as further evidence that the heating resulted from biological activity in the samples and not from faulty adjustment of the control system.

Since the control mechanism must operate at high efficiency over extended periods of time, it is necessary to keep a constant check on its operation. This can be done by carefully noting the exact position of the light beam on the photoelectric cell when the relay cuts in and out and when the galvanometer is at zero setting. When readings are being taken these points can be checked and any appreciable deviations indicate a weakening in a tube or in the light source. When this occurs the exhausted unit can be replaced before a complete failure of the control results. In a similar manner the light bulbs used as heaters must be changed at regular intervals. Through observance of these simple precautions it was possible to operate the unit almost continuously for six months without a single failure of the control mechanism.

Materials

A sample of 1 C.W. flax produced at Indian Head, Sask., in 1943 was selected for study. It was thoroughly cleaned to remove trash, weed seeds, and broken kernels after which it showed an oil content of 41.8% and a bushel weight of 54.0 lb.

The sunflower seed was from a lot of 1 C.W. Mennonite grown in 1943 at Borden, Sask. After thorough cleaning to a dockage-free basis, it showed an oil content of 28.0% and a bushel weight of 29.0 lb. In addition to studies on the whole seed a single run was made on a portion of this material dehulled immediately prior to the test.

Results

The results obtained in this study are all presented graphically and the individual points represent actual recorded temperature readings. This method of presentation has been adopted to illustrate changes in the rate of heating with time. It will be noted from the figures that all points fall on regular, smooth curves and the temperature at any given time can readily be estimated.

The Nature of Heating

Three samples of material at moisture contents that would definitely ensure heating were selected to illustrate the nature of the relation between rate of heating and time. These were (i) flaxseed at 14.7% moisture, (ii) whole sunflower seed at 15.2% moisture, and (iii) dehulled sunflower seed at 11.2% moisture. The curves for the first two of these samples are shown in Fig. 1, while that for dehulled sunflower seed is shown in Fig. 2. It will be

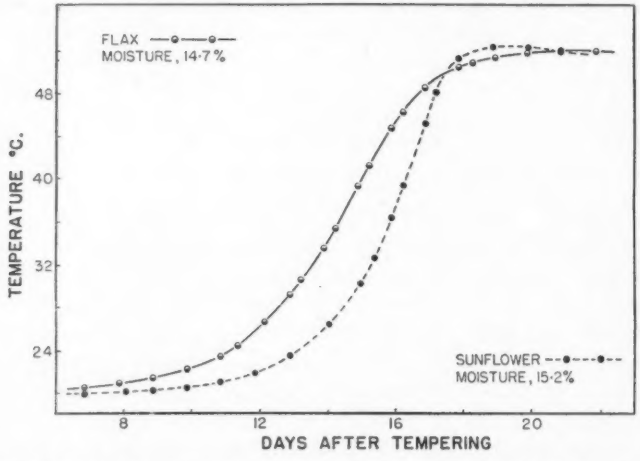

FIG. 1. *The relation between temperature attained and time for flax and whole sunflower seed under adiabatic conditions.*

noted that all three curves are essentially similar in shape, but differ with respect to the length of the induction period and the acceleration of the rate of heating with the lapse of time. All three samples were started at temperatures between 19.5° and 21° C. and after induction periods of seven days for flaxseed, nine days for whole sunflower seed, and five days for dehulled sunflower seed the temperatures began to rise and reached a maximum of 51° to 52° C. At this point no further increase in temperature occurred. In flaxseed the maximum temperature, 52.1° C., was reached in 20 days and after an additional 10 days the temperature had fallen back to 51.5° C.

If these data are compared with respiration data previously reported by Larmour, Sallans, and Craig, it will be noted that at corresponding moisture

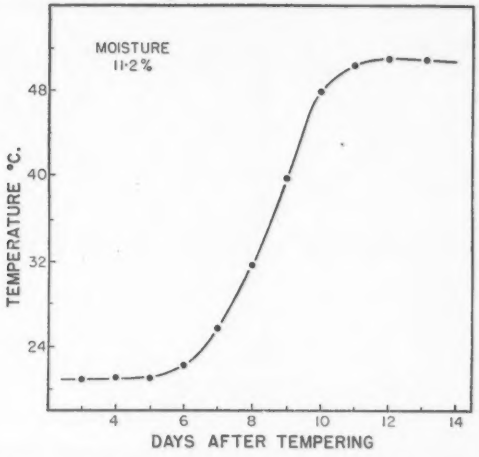

FIG. 2. *The relation between temperature attained and time for sunflower meats under adiabatic conditions.*

levels, the induction periods for active heating are longer than those for the development of an accelerated respiration rate. This observation applies equally well to the data in Figs. 3 and 4 of the following section. This furnishes evidence that an accelerated, over-all respiration rate precedes active heating.

The moisture levels used in the present investigation and in the work on respiration are definitely below those required for active germination of the seeds under study. Hence, it is reasonable to assume that under these conditions the respiration of the embryo of the seeds would be virtually constant for any given moisture level and that the observed acceleration in the over-all respiration rates must be due to the active growth of fungi and bacteria on the grains. According to this interpretation the low respiration rate of grain during the induction period would approximate the true respiration rate of the grain due to embryonic activity and the accelerated respiration rate would represent active proliferation of the microflora. Furthermore, it follows that the low respiratory activity of the embryo is not sufficient to generate enough heat to cause a temperature rise under almost perfect adiabatic conditions. These considerations make it appear that heating of grains in commercial storage must result from active growth of the microflora rather than from embryonic activity.

It will be noted that all three curves show maximum temperatures of 51° to 52° C. While no attempt was made to isolate and classify the fungi and bacteria present on the grains, it is well-established that the thermal death point of such genera as *Penicillium* and *Aspergillus* is between 50° to 55° C. Therefore, it is reasonable to assume that organisms of this type cause the first stage in the heating of grain. It should be noted in this connection that

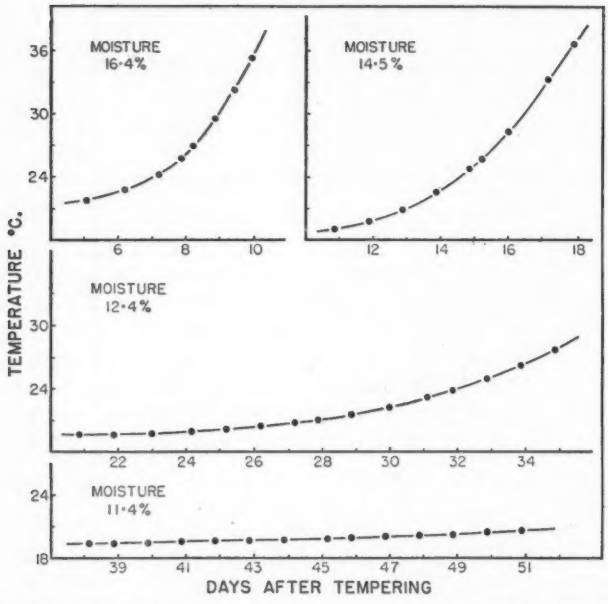

FIG. 3. *The relation between temperature attained and time for flax of different moisture levels under adiabatic conditions.*

Ramstad and Geddes (6), using a similar adiabatic thermostat in their study on soybeans, report temperatures as high as 80° C. Furthermore, their data show almost linear relations between time and temperature with no apparent break at the thermal death point of the common fungi. It is difficult to understand how temperatures as high as 80° C. can be produced except by by thermophilic bacteria. If this is the case an inflection point in the curve would be expected at 50° to 55° C. corresponding to the thermal death point of the common fungi and the subsequent development of thermophilic organisms. The absence of an inflection point in the data of Ramstad and Geddes might be explained on the basis of a restricted air flow and a resulting slow temperature rise through the point where the common fungi are killed and the thermophilic organisms proliferate actively. However, under the conditions of the present investigation no evidence of heating above the thermal death point of common fungi was obtained.

Estimation of Safe Moisture Limit for Storage

The application of the adiabatic thermostat to the determination of safe moisture limits for the storage of grain is a slow procedure since only a single sample can be run at one time. However, it has been observed that, as the moisture level approaches the limiting value, the induction period for the development of heating lengthens. Taking advantage of this fact, a number of samples having progressively decreasing moisture levels were tempered at

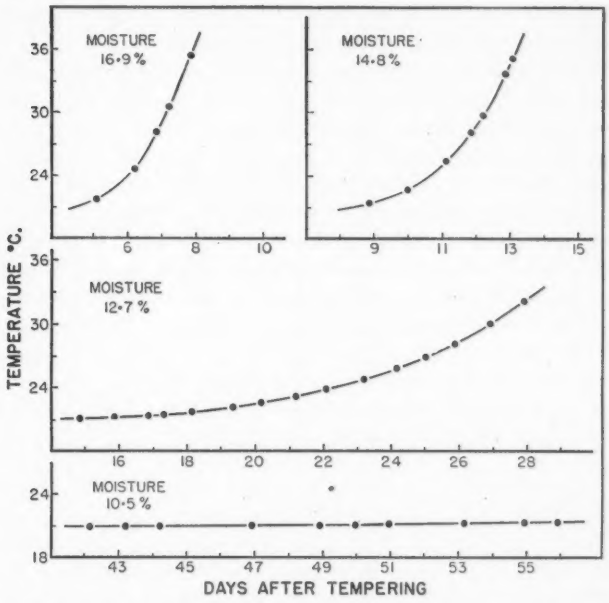

FIG. 4. *The relation between temperature attained and time for whole sunflower seed of different moisture levels under adiabatic conditions.*

the same time and stored in thermos bottles in the same room as the thermostat. The sample of highest moisture content was placed in the thermostat and as soon as definite heating occurred it was removed and the next sample, which had not yet passed through its induction period, was placed in the thermostat. In this manner a series of samples can be run in succession and the total time can be materially reduced. This procedure was followed and the air in the samples not in the thermostat was changed once a day by means of an aspirating bulb.

Data obtained on flaxseed at moisture levels of 16.4, 14.5, 12.4, and 11.4% are shown in Fig. 3. At the end of each run the samples were removed from the apparatus and the moistures were found to be 16.6, 14.6, 12.4, and 11.3%, respectively. A similar set of data on whole sunflower seed at moisture levels of 16.9, 14.8, 12.7, and 10.5% are shown in Fig. 4. The moistures at the end of this experiment were 17.2, 14.8, 12.6, and 10.4%, respectively. It is evident from Fig. 3 that flaxseed at a moisture content of 11.4%, just 0.9% above the limit for 'straight grade,' shows a definite indication of heating. The actual temperature rise was from 19.5° C. to 20.7° C. in a period of 51 days of storage. In sunflower seed at 10.5%, just 1.0% above the moisture limit set for this grain, there was a slight temperature rise from 20.6° C. to 21.4° C. over a period of 56 days.

A comparison of the first three curves for flaxseed with the corresponding ones for sunflower seed, at approximately the same moisture levels, indicates that the sunflower seed has a shorter induction period and shows a more rapid rate of heating than flaxseed. This would suggest that the ultimate moisture limit at which sunflower seed would show no heating is lower than that for flaxseed. In the bottom curves of Figs. 3 and 4 it will be noted that the sunflower seed was 0.9% lower in moisture content than the flaxseed and still a slight indication of heating was obtained. Hence, if 10.5% is the accepted safe limit for flaxseed, it would appear that a value of 9.5% is none too low for sunflower seed especially if storage periods of a year or more are considered. Theoretically, if a sufficient number of runs were made covering longer periods of time, it should be possible to reach a closer approach to the actual value set for flaxseed. However, there appears to be little point in carrying these tests to the ultimate limit on single samples since variations in oil content and in the "soundness" of the sample would affect the ultimate limit attained. The importance of the present data lies in the fact that actual heating of these grains has been demonstrated, at moisture levels within 1% of those set for grading purposes, even under comparatively short storage periods of less than two months. This furnishes definite evidence that, for longer storage under varying conditions of temperature and 'soundness' of the grain, the margin of safety under present grading specifications is very small.

Discussion

From the preceding sections it would appear that spontaneous heating in grain must be attributed to active proliferation of its microflora. If the moisture level is such that these organisms can grow, then heating will result. Confirmation of this hypothesis may also be drawn from the data on the hygroscopic equilibrium of sunflower seed (4), flaxseed (2, 4), soybeans (4, 6), and wheat (2). From these data, if 14.5% moisture is taken as the critical value for wheat, it is found that this corresponds to a relative humidity of approximately 74% in the interstitial air in bulk wheat under equilibrium conditions at 25° C. If an estimate of the moisture content of sunflower seed, flaxseed, and soybeans is made, under these conditions, it is found that the values fall very close to the generally accepted limits set for the safe storage of these grains. Therefore, it would appear that the common critical factor in the phenomenon of the spontaneous heating of grains is the relative humidity of the interstitial air in the samples. More specifically the fungus spores on the samples must reach a certain moisture content before they will germinate and grow, and this moisture level appears to be in equilibrium with air at a relative humidity of approximately 74%.

The differences between values obtained from laboratory studies and from commercial experience, which amount to 1.5% using the respiration technique and 1.0% using the adiabatic thermostat, may result from a number of causes. It is not inconceivable that, if these methods were refined to permit accurate control of the moisture level of the samples over long periods of time,

values more closely approaching those obtained from commercial experience would be obtained. However, there are marked differences between the conditions existing in small samples and those in large storage bins. In the laboratory the sample, being small, tends to have a uniform temperature throughout, while in a larger bulk there are differences in temperature between various sections of the bin that result in the setting up of convection currents in the bulk of the grain (6). The effect of temperature differential on moisture content has recently been studied by Anderson, Babbitt, and Meredith (1). From their results it is evident that, even though grain is stored at a moisture level that would normally preclude the possibility of heating, it would be possible to obtain regions in a bin where the moisture content would rise to dangerous levels and heating might occur. It is obviously impossible to study this possibility by means of the respiration technique and in the adiabatic method the samples are small and the temperature so uniform that convection currents are reduced to a minimum.

In addition to these limitations, laboratory studies have been confined to thoroughly cleaned 'sound' grain while in commercial channels it is often necessary to store the grain, as received, for considerable periods of time. When uncleaned grain is 'spouted' into a bin, the trash, being lighter than the grain, tends to collect in the near side of the bin. Commercial operators have observed that when 'heating' occurs, it usually starts in this accumulation of trash and then spreads to the remaining sections of the bin. This may be due to a greater infection in the trash, but it seems equally probable that it is related to the moisture content. It has been observed in this laboratory that uncleaned samples of sunflower seed usually have a higher moisture content than the same samples after cleaning. This indicates that the actual moisture level of the trash is higher than that of the grain itself. The spreading through the remainder of the bin could be explained on the basis of convection currents causing a redistribution of moisture. Furthermore, unpublished data obtained in this laboratory indicate that as active heating progresses there is an apparent increase in the moisture level of grain. This results partly from the loss of dry matter in the form of carbon dioxide without a compensating loss of moisture and also from the accumulation of moisture and volatile products produced by the respiration of the grain and its microflora. It is, therefore, evident that once active heating starts in a localized portion of a bin convection currents and the production of moisture by the 'heating' will result in a spread of the 'hot spot.'

From these considerations it is obvious that the results of small-scale laboratory studies cannot be interpreted directly in terms of commercial storage conditions. They may be used to arrive at an approximation of the limiting safe moisture limit for the storage of grains, but it would appear that, regardless of the method used or the values obtained, some empirical correction must be applied to compensate for effects in large bins that cannot be duplicated in the laboratory.

Acknowledgment

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FLAVOUR REVERSION IN HYDROGENATED LINSEED OIL

I. THE PRODUCTION OF AN ISOMER OF LINOLEIC ACID FROM LINOLENIC ACID[1]

By H. W. Lemon[2]

Abstract

Linseed oil that has been hydrogenated to a plastic consistency is subject to a type of deterioration termed "flavour reversion" when heated to temperatures used in baking or frying. Investigation of the course of hydrogenation of linseed oil by the spectral method of Mitchell, Kraybill, and Zscheile (11) has indicated that linolenic acid is converted to an isomeric linoleic acid; this acid differs from naturally occurring linoleic acid in that the double bonds are in such positions that diene conjugation is not produced by high-temperature saponification. In a typical hydrogenation, the concentration of the isomeric acid increased to a maximum, at about iodine number 120, of 18% of the total fatty acids, and at iodine number 80, at which point the plasticity was similar to that of a commercial shortening, the concentration of the isomer was 13%. Evidence is presented that the isomeric linoleic acid in partially hydrogenated linseed oil is responsible for the unpleasant flavour that develops when the oil is heated.

The shortage of vegetable oils that developed in Canada after the outbreak of war in the Pacific has made it advisable to investigate the possibility of using linseed oil in the manufacture of shortenings, as it is the only vegetable oil that is produced in this country in large quantities. Linseed oil can be hydrogenated easily, but it requires considerably more hydrogen than the oils normally used. However, the greatest obstacle in the way of utilizing hydrogenated linseed oil in shortenings is that it is subject to a type of deterioration termed "flavour reversion." When freshly deodorized, it is reasonably bland, but it develops an off-flavour on storage, and a characteristic, unpleasant odour when it is subjected to baking or frying temperatures. In connection with investigations undertaken to determine the effect of different conditions of hydrogenation on flavour reversion, to be published later, some evidence was obtained indicating that a hydrogenation product of linolenic acid contributed to this reversion. This evidence is presented in the present article.

The problem of flavour reversion is well known in the edible oil industries. Fish oils and soybean oil are subject to it, but to a lesser extent than linseed oil. Flavour reversion in soybean oil has been ably discussed by Bickford (3), and he has reviewed the theories that have been proposed to explain the phenomenon. Some evidence has been put forth that a non-glyceride constituent of the oil is the cause of the trouble. Others (7) suggest that linolenic acid is in some way responsible for it, and the fact that the tendency to flavour reversion in linseed and soybean oils is roughly proportional to their linolenic acid content lends some support to this theory.

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[2] *Research Fellow.*

It has been suggested that the development of an unpleasant flavour in partially hydrogenated linseed oil may be due to linolenic acid that has escaped hydrogenation. The glycerides of linseed oil contain 30 to 60% of linolenic acid and 10 to 25% of linoleic acid. When an oil is hydrogenated, the rise in melting point is due to the formation of both saturated fatty acids and "iso-oleic acid," which is a solid isomer of oleic acid. If the reaction is selective, the most unsaturated fatty acids are largely converted to less unsaturated acids before the latter become completely hydrogenated. Selectivity is influenced by the operating variables, but, as Bailey, Feuge, and Smith (1) have pointed out, the formation of iso-oleic acid is favoured by each of the conditions that contribute to selectivity. Therefore, linseed oil, since it contains such a large proportion of linolenic and linoleic acids, may become too hard on hydrogenation before these highly unsaturated acids have disappeared. It was decided that the change in fatty acid composition that takes place as hydrogenation proceeds should be accurately determined, with the hope that it might provide a clue to the cause of flavour reversion.

Experimental

Procedure

Raw linseed oil was refined with an aqueous solution of sodium hydroxide, containing $4\frac{1}{2}$ times the quantity of alkali required for neutralization of the free fatty acids, then bleached with an activated earth. The refined oil was hydrogenated in a vertical cylindrical steel pressure vessel, with a capacity of about 2 lb. of oil, which was equipped with an agitator of the paddle type, cooling coil, and thermometer well. Electrolytic hydrogen was supplied from a cylinder through a pressure regulating valve and a tube extending through the top of the vessel into the oil. Heat was supplied by means of a ring gas burner underneath the vessel. A variety of temperatures and pressures have been employed, and both commercial and laboratory prepared catalysts were used. The laboratory catalysts were of two types, (*a*) nickel formate reduced in oil at 265° C. in an atmosphere of hydrogen, and (*b*) nickel carbonate reduced dry at 400° C. in an atmosphere of hydrogen. After the conclusion of each hydrogenation, the catalyst was removed by filtration, and the hydrogenated oil was deodorized with steam for three hours at 200° C. and a pressure of 2 to 4 mm. of mercury.

Methods of Analysis

The Kaufmann (10) method of analysis was first applied to hydrogenated linseed oil samples. This method entails the determination of the iodine and thiocyanogen numbers, and the saturated acid content of the sample, and from these values oleic, linoleic, and linolenic acid percentages are calculated. The fatty acid composition of various linseed oils has been determined by Rose and Jamieson (15) and by Painter and Nesbitt (12) in this way. Similar results were obtained for the linseed oil used in the hydrogenation experiments, but when the method was applied to hydrogenated linseed oil, the calculated values for the unsaturated fatty acids were impossible, as the linoleic acid

values were consistently negative over a wide range of iodine numbers when the constants suggested by Riemenschneider, Swift, and Sando (13) were used. The results indicated that some product of the hydrogenation did not absorb thiocyanogen in the expected manner.

A new method of fat analysis has been developed by Mitchell, Kraybill, and Zscheile (11) that utilizes the fact that fatty acids containing conjugated double bonds show characteristic absorption bands in the ultra-violet region of the spectrum. Conjugation is achieved by saponification of the fat sample at 180° C. with a potassium-hydroxide–ethylene-glycol solution. Conjugated linoleic acid has an absorption maximum at 2340 Å, while conjugated linolenic acid has maxima at 2340 Å and at 2680 Å and smaller maxima at 2580 Å and 2790 Å. The specific absorption at 2340 Å and 2680 Å has been determined for the pure acids with the use of a spectrophotometer; the values are reproducible when the alkali treatment is standardized. If the specific absorption at 2340 Å and 2680 Å of the sample to be analysed is determined, the concentration of linolenic and linoleic acids can be calculated by comparison of the absorption with that of the pure acids. Very small quantities of linolenic acid can be detected and measured in this way.

The spectral method of analysis has been applied to hydrogenated linseed oil, in conjunction with the determination of saturated and iso-oleic acids by the lead salt precipitation method of Baughman and Jamieson (2). A Beckman spectrophotometer (6) was used for the absorption measurements.

Results

Results of Spectral Analysis

The specific absorption measurements at 2680 Å showed that linolenic acid disappeared rapidly on hydrogenation of linseed oil. It was expected that there would be a corresponding increase in linoleic acid during the initial stages of hydrogenation, but absorption measurements at 2340 Å indicated, on the contrary, that the linoleic acid content became steadily less.

The percentages of oleic and saturated acids were calculated from the iodine numbers of the samples and from the values for linoleic and linolenic acids that were obtained by spectral analysis. The calculated oleic acid values were much higher than is usual in hydrogenated oils, and the values for saturated acids were considerably lower than those arrived at by the lead salt method. As an example, a hydrogenated linseed oil with iodine number 88 contained 2.4% linolenic acid and 2.7% linoleic acid, as determined by spectral analysis; total oleic acid was calculated to be 89.8% and saturated acids 5.1%. Such values are highly improbable, as the value for saturated acids obtained by the lead salt method was 20.5%.

Linoleic and oleic acid percentages can be calculated if the iodine number of the sample, and the values for saturated, iso-oleic and linolenic acids, as determined by the lead salt method and by spectral analysis, are known. When this was done for the above sample, the linoleic and oleic acid values were 17.8 and 44.2%, respectively. It was suspected that the reason for the

difference between the calculated value for linoleic acid and that arrived at by spectral analysis is that hydrogenation of linolenic acid produces an isomeric linoleic acid in which the double bonds are in such a position that a conjugated system is not formed on treatment with potassium hydroxide at 180° C.

Isolation of Isomeric Linoleic Acid

In order to prove the presence of an isomeric linoleic acid in partially hydrogenated linseed oil, a quantity of the separated fatty acids of iodine number 82.1 was dissolved in acetone, and subjected to the low temperature crystallization method for purification of linoleic acid described by Brown *et al.* (4, 5, 8, 9). Most of the saturated and oleic acids crystallized, and were removed by inverted suction filtration. Approximately 12% of the original acids remained in solution, and, after removal of the acetone, the residue was distilled *in vacuo*. The iodine number of the distillate was 152.1 and, if it is assumed that it is a binary mixture of oleic and linoleic acids, the concentration of the latter would be 68%.

Fatty acids separated from sunflower seed oil of iodine number 134.0 were treated in a similar manner to obtain a natural linoleic acid concentrate for comparison with that separated from hydrogenated linseed oil. The distilled concentrate had an iodine number of 162.6, corresponding to a linoleic acid concentration of 80%.

The two fractions were heated with the ethylene-glycol–potassium-hydroxide reagent of Mitchell, Kraybill, and Zscheile, and spectral absorption measurements were made on the soap dissolved in absolute alcohol. Absorption is expressed as "specific alpha."

$$\text{Specific } \alpha = \frac{\log_{10}\frac{I_0}{I}}{cl}$$

where α = absorption coefficient,
I_0 = intensity of radiation transmitted by the solvent,
I = intensity of radiation transmitted by the solution,
c = concentration of solute in grams per 1000 ml.,
l = length in centimetres of solution through which the radiation passes.

The absorption curves for the linoleic acid concentrates from sunflower seed oil and from hydrogenated linseed oil are shown in Fig. 1, Graph *A*. Curve 1 for natural linoleic acid shows the characteristic absorption maximum at 2340 Å due to diene conjugation, with a specific α value of 76.7. Mitchell, Kraybill, and Zscheile found the specific α value for pure linoleic acid to be 87.1. In comparison, the absorption at 2340 Å due to the linoleic acid concentrate from hydrogenated linseed oil, as shown in Curve 2, is small. Curve 2 has been replotted in Graph *B* with the specific α scale expanded 10 times in order to show more clearly the maximum in the region of 2340 Å, and

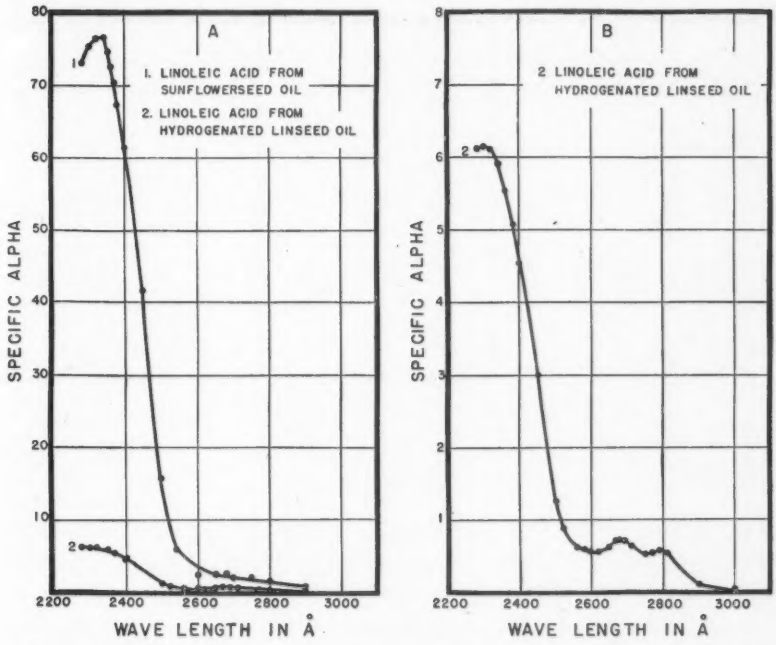

FIG. 1. *Absorption spectra of linoleic acid concentrates from sunflower seed oil and from hydrogenated linseed oil. Graph B is a replot on an enlarged scale of Curve 2 of Graph A.*

smaller maxima at 2680 Å and 2790 Å. The maximum near 2340 Å is probably caused by the presence in the distilled concentrate of a small amount of linoleic acid from the linseed oil that has escaped hydrogenation. The maxima at 2680 Å and 2790 Å are typical of triene conjugation, and indicate the presence of a trace of linolenic acid in the concentrate.

These results prove beyond doubt that hydrogenation of linolenic acid causes the formation of an isomeric linoleic acid that differs from natural linoleic acid in that the double bonds are so arranged that they will not form a conjugated system.

The Course of Hydrogenation of Linseed Oil

The change in fatty acid composition that occurs during a typical linseed oil hydrogenation is shown graphically in Fig. 2. The oil was hydrogenated at 140° C., 25 lb. gauge pressure, using an oil-reduced nickel formate catalyst in a concentration equivalent to 0.2% of nickel, based on the weight of oil. Samples were taken periodically. Saturated acids and iso-oleic acid were determined by the Baughman and Jamieson method, linolenic and linoleic acids by spectral analysis. Oleic and total linoleic acids were calculated in the way already described, and the values for isomeric linoleic acid (this acid will be termed "iso-linoleic acid") were obtained by subtracting the

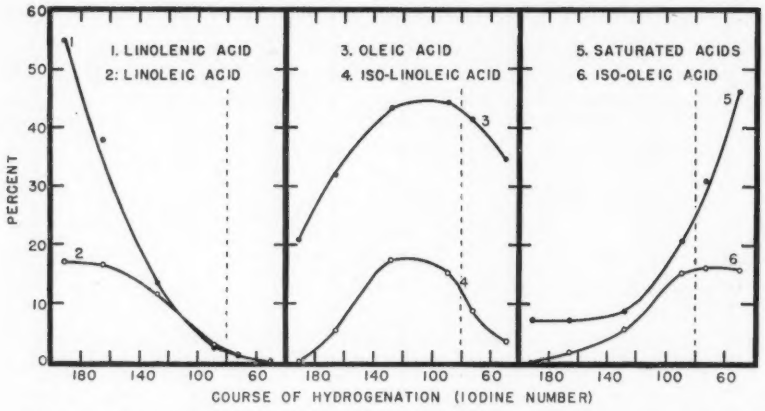

FIG. 2. *Progressive change in fatty acid composition on hydrogenation of linseed oil. The broken vertical lines at iodine number 80 mark the point at which the consistency of the oil was similar to that of a shortening.*

figures for linoleic acid indicated by spectral absorption from the calculated values for total linoleic acid.

The hydrogenation of the linolenic and linoleic acid fractions of the linseed oil was initially rapid, and was accompanied by marked increases in iso-linoleic, oleic, and iso-oleic acids, but by only a small increase in saturated acids. The concentrations of oleic and iso-linoleic acids were greatest at about iodine number 120. Further hydrogenation caused a rapid increase in saturated acids with corresponding decreases in oleic and iso-linoleic acids; during this phase of the reaction linolenic acid was hydrogenated more slowly. Hydrogenated oil of iodine number between 80 and 85 had a melting point similar to that of a commercial shortening, but there still remained traces of linolenic and linoleic acids, and about 14% of iso-linoleic acid.

The fatty acid compositions of a number of partially hydrogenated linseed oils, prepared from the same bulk of linseed oil, are given in Table I. Some of the oils contained traces of linolenic acid, particularly those hydrogenated at low temperature. All the samples contained considerable quantities of iso-linoleic acid. The analyses of Samples 105, 99, and 110 show the effect of hydrogenation temperature on the fatty acid composition. Increasing the temperature caused a decrease in the production of saturated acids, and an increase in iso-oleic acid. The effect of temperature increase on the iso-linoleic acid content of the hydrogenated oil was not very great.

Role of Iso-linoleic Acid in Flavour Reversion

It was suspected that the iso-linoleic acid content of a hydrogenated linseed oil shortening may be responsible for the characteristic and unpleasant flavour that develops in the shortening when it is heated. Strong, though

TABLE I

FATTY ACID COMPOSITION OF HYDROGENATED LINSEED OIL SAMPLES

Number	Temperature of hydrogenation, °C.	Iodine number	Saturated acids, %	Iso-oleic acid, %	Oleic acid, %	Linoleic acid, %	Iso-linoleic acid, %	Linolenic acid, %
105	115	80.3	23.9	11.2	48.6	1.4	14.1	0.8
99	140	81.9	20.4	16.8	47.3	0.6	14.9	0
110	190	80.1	19.4	23.6	44.7	0.2	12.1	0
85	140	76.1	26.1	13.7	46.0	0	13.9	0.3
102	140	76.6	24.1	15.8	47.0	0.4	12.7	0

Numbers 105, 99, 110 hydrogenated with a commercial catalyst.
Number 85, hydrogenated with oil-reduced nickel formate catalyst.
Number 102, hydrogenated with dry-reduced nickel carbonate catalyst.

not entirely conclusive, evidence for this has been afforded by the following experiments:—

(1) When samples taken throughout a linseed oil hydrogenation were heated under standardized conditions and then scored for odour development, it was found that the linseed oil odour disappeared early in the hydrogenation, but was replaced by the characteristic odour of hydrogenated linseed oil. The intensity of this odour was at a maximum in samples with iodine numbers between 80 and 130; it decreased on further hydrogenation, and was very slight in samples with iodine numbers less than 50. In other words, the intensity of the odour roughly paralleled the accumulation and disappearance of iso-linoleic acid.

(2) Partial hydrogenation of pure methyl linolenate, prepared according to the method of Rollett (14), yielded a product that, when heated, developed an odour similar to that of heated hydrogenated linseed oil, but quite different from the odour of the unhydrogenated ester or from that of pure methyl linoleate, when treated in the same way.

(3) When the distilled iso-linoleic acid concentrate from hydrogenated linseed oil was heated, a very strong odour developed; this was similar to the odour of heated hydrogenated linseed oil.

Discussion

The double bonds in linolenic acid are in the 9 : 10, 12 : 13, and 15 : 16 positions, and in linoleic acid they are in the 9 : 10 and 12 : 13 positions. Van der Veen (16) subjected hydrogenated methyl linolenate to ozonolysis, and concluded from the nature of the breakdown products that hydrogenation caused saturation of the 12 : 13 double bond, yielding a 9 : 10, 15 : 16 methyl linoleate. The results of the spectral absorption measurements on the isomeric linoleic acid from hydrogenated linseed oil support this conclusion,

as double bonds in the 9 : 10 and 15 : 16 positions are too far apart to form a conjugated system on treatment of the oil with alkali.

It has not been definitely established that iso-linoleic acid is the cause of the development of the unpleasant flavour in hydrogenated linseed oil, but there is no doubt that the responsible substance is destroyed by hydrogenation as the difficulty is largely overcome by continuing the hydrogenation until the iodine number is lower than 50; such a product, however, is too hard for use as a shortening. To make an acceptable linseed oil shortening, the substance causing the development of flavour should be hydrogenated completely, but the formation of saturated and iso-oleic acids, which is responsible for the rise in melting point, should be greatly suppressed. To achieve this, a hydrogenation catalyst is required that combines the properties of both exceptionally high selectivity and good iso-oleic acid suppression.

Acknowledgments

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RESIN–RUBBER FROM CANADIAN GROWN PLANTS

I. VARIATION IN SELECTED GENERA[1]

By R. W. Watson[2] and N. H. Grace[2]

Abstract

The common milkweed, *Asclepias syriaca* L. was selected from the most promising native genera as the only species with a resin–rubber content high enough to warrant extraction studies. Rubber is concentrated in the leaves. Drying milkweed under glass in air and full daylight at temperatures up to 60° C. does not reduce the rubber content. Concentrates are produced by fermentative decomposition. There is a gradual increase in the leaf rubber content during the growing season, with a maximum of about 3.5% in late September in the Ottawa district.

Introduction

This communication describes results of preliminary investigations of the resin–rubber content of native Canadian plants. While the main object of the research was the development of simple mechanical extraction methods, it was first necessary to select suitable species with which to work.

Much has been done, chiefly in the United States and in Russia, within the past two decades in attempts to select and develop temperate-climate plants as sources of natural rubber. When the present rubber shortage was foreshadowed early in 1942 a co-operative program was initiated in several Canadian laboratories.* The virtual non-existence of previous investigation of this kind in Canada and the urgency of the project made it necessary to restrict analytical studies to the most promising genera.

Species of three native generic groups, namely, *Asclepias*, *Solidago*, and *Apocynum* have been considered by various investigators as commercial sources of rubber. There is an extensive literature dealing with *Asclepias*, which has been thoroughly reviewed recently by Whiting (17) and Rheineck (15). After a careful study of 20 species of milkweed introduced into the U.S.S.R., Medvedev (11) concluded that the common North American milkweed *A. syriaca* L. was the best all-round choice as a source of rubber. *Solidago* was favoured by the late T. A. Edison. After completing an extensive survey of rubber-bearing plants capable of being grown in the United States, Edison (1) selected *Solidago leavenworthii* Torr. as especially worthy of further investigation. The range of this species does not extend into Canada. However, Polhamus (13) has reported rubber contents of 1.0 to

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[2] *Biochemist, Industrial Utilization Investigations.*

* *The Department of Botany, University of Toronto, the Central Experimental Farm and Science Services, Dominion Department of Agriculture, and the National Research Council Laboratories, Ottawa.*

6.4% in southern members of goldenrod species that are native to Canada. The collections analysed by Polhamus were obtained from Washington, D.C. southward. Similarly both in the United States (3, 6) and in Russia (9, 12) species of *Apocynum* have been found to contain from 0.5 to 8.5% of rubber in their leaves. In Russia one at least of these species is reported to have been cultivated (9). Tropical and subtropical species of *Euphorbia* have high rubber contents, but native Canadian spurges are small plants having low percentages of rubber. Several other native genera, viz., *Lactuca*, *Sonchus*, *Tragopogon*, *Papaver*, and *Campanula* occupy a secondary position in the literature. In the main they are reported to contain about 1% or less of rubber.

Materials and Methods

Periodic collections of *Asclepias*, *Solidago*, and *Apocynum* were made by the Experimental Farms and Science Services of the Department of Agriculture and passed to the National Research Council in the dried condition for analysis. Collections of *Apocynum androsaemifolium* L., the common dogbane, were drawn from four belts—Atlantic (Nova Scotia), Eastern (Ontario), Central (Saskatchewan), and Pacific (British Columbia). Specimens were taken at one or more stations in each of these regions, viz., Kentville and Highbury, N.S.; Ottawa, Ontario; Saskatoon, Sask.; and Summerland, B.C. Collections of the common milkweed and of a few species of goldenrod were confined to Eastern Canada. A collection of *Asclepias sullivantii* Engelm. from Walpole Island, Lambton County, Ontario, on July 20, 1943, was submitted by Dr. W. Sherwood Fox and Mr. E. H. McKone. Preliminary work had indicated that the common milkweed might be the most promising native plant in the Ottawa district so that numerous collections weighing 100 to 2000 lb. (fresh weight) were made by the authors from early summer 1942 onward (5). Similar collections were made during the summer of 1943.

The smaller July and early August collections were dried in tunnel or box driers at 45° to 60° C. Plants for comparison were dried at room temperature. The large late August and September collections were dried at 30° to 40° C. on greenhouse benches. Analyses of milkweed leaves spread thinly and exposed for weeks to full daylight in the greenhouse were compared with others of the same collections dried in the dark. The rubber contents of leaves at various heights on the plant were determined.

Samples for analysis were obtained from the mechanically mixed entire ground mass of leaves from the smaller collections. The leaves were removed after drying and ground in a Wiley mill, one passage through the mill sufficing to reduce about 75% of the mass to pass 30 mesh. Plants for analysis from the larger collections were chosen at random, and the leaf powders prepared in the same way.

Soxhlet and Bailey–Walker extractors were used, and their efficiency compared by quadruplicate analyses of thoroughly mixed leaf powders from two similar early August collections from the same site. The variations

introduced by the use of cellulose and alundum thimbles in the former and of Gooch crucibles, Jena filter crucibles, and siphon cups in the latter were determined. Two- to five-gram samples of mixed ground tissue were placed in a thimble lined with filter paper and closed with a cap of extracted cotton wool. Twenty-four-hour periods each for acetone and benzene extraction were adopted as standard after preliminary studies. Following acetone extraction the thimble was air-dried for four hours at 60° C.

Bailey–Walker extractors gave fairly consistent results only when siphon cups were used. Variable results were obtained by packing the base of the cup with cotton wool, partly filling with leaf powder mixed with ignited quartz sand, and capping with cotton wool. When siphoning was continuous, channelling occurred, and the extraction was less complete. Moreover, the solvent usually boiled in the siphon cups, with resultant loosening of the cotton-wool plugs and escape of powder particles. Cellulose thimbles lined with filter paper proved superior to those of alundum in both Soxhlet and Bailey–Walker extractors.

Data from quadruplicate analyses of milkweed leaf powders using Soxhlet and Bailey–Walker extractors are given in Table I. The coefficients of variation with Bailey–Walker extractors are tripled compared with those obtained by the use of Soxhlets. This is a typical example of comparative results with these two types of apparatus, and hence Soxhlets were used in most of the subsequent analyses.

TABLE I

COMPARATIVE RESULTS WITH SOXHLET AND BAILEY–WALKER EXTRACTORS

——	Soxhlet		Bailey–Walker	
	Per cent extraction with			
	Acetone	Benzene	Acetone	Benzene
Mean*	16.93	1.89	12.47	2.25
Standard deviation	0.12	0.03	0.28	0.09
Coefficient of variation, %	0.7	1.5	2.2	4.2

* *Means are from leaf collections from the same site six days apart.*

In duplicate analyses, omission of the drying step after acetone extraction led to a substantial increase in the benzene extract. This may be attributed to continued extraction by residual acetone or to the effect of the mixed solvents.

The iodine numbers (8) of evaporated benzene extracts range from 331 to 332. The percentage rubber hydocarbon in the extracts is therefore approximately 89. These values check closely with those obtained (2) by chromic acid oxidation analyses.

A moisture determination was made simultaneously on a duplicate sample by drying *in vacuo* for 16 hr. at 105° C. All percentages are expressed on a dry-weight basis. The acetone extract is hereafter referred to as "resin" though it has been determined that the 24 hr. period is inadequate for complete extraction. The benzene extract is designated "rubber".

Results

Drying

Milkweed leaves of a mid-August collection were dried at temperatures of 20° to 25° C. and 45° to 60° C. Resin and rubber percentages, respectively, were 10.04 and 1.23 at the lower temperature, and 9.97 and 1.18 at the higher. Such results showed that not only was the temperature range of 45° to 60° C. adequate to dry the leaves in 24 hr., but it did not affect quantitatively the resin and rubber content.

A dozen plants sampled at random from a late July collection were dried immediately in the dark as controls. The remainder was spread on a greenhouse bench exposed to full daylight, and a dozen plants taken at random were removed at weekly intervals. As shown in Table II, the percentages of rubber declined slightly during the period of exposure. In contrast to the effect of light on the rubber content of *Solidago* leaves (14), exposure to solar radiation filtered through window glass appears to diminish slowly the percentage benzene extract of *Asclepias* leaves.

TABLE II

EFFECT OF EXPOSURE TO LIGHT ON RESIN–RUBBER CONTENT OF MILKWEED LEAVES

Period of exposure, weeks	Acetone extract, %	Benzene extract, %
0	11.1	1.1
1	7.7	1.1
2	11.7	1.2
4	11.0	0.9
5	11.5	1.0
7	11.1	0.9

Fermentative Decomposition

The effects of fermentative decomposition may be seen from the analyses of three random samples of a late July collection given in Table III.

The apparent increase in rubber content as well as the variability in the triplicate analysis, due presumably to sampling errors, is typical of the results obtained when marked fermentative decomposition occurs. It appears likely that the entire increase in rubber content relates to the decrease in the dry weight of the leaves on fermentation.

TABLE III

EFFECTS OF FERMENTATIVE DECOMPOSITION ON RESIN–RUBBER CONTENT OF MILKWEED LEAVES

Treatment	Acetone extract, %	Benzene extract, %
Whole plants dried at 45° to 50° C.	—	1.2
Leaves off after drying	15.7	1.1
Whole plants sprinkled with water daily for	13.3	2.1
seven days on a greenhouse bench, then	13.7	2.6
dried at 45° to 50° C.	10.4	2.5

Resin–Rubber Contents of Selected Species

Dogbane

The common dogbane, *Apocynum androsaemifolium*, probably contains no rubber in the roots and little in the stems (average 0.3%). Analyses of 25 leaf samples collected from early June to mid-September from stations across Canada show a maximum rubber content of 1.2%. The resin content varies from 11 to 27%, depending in part on the developmental stage. There is a tendency for the rubber content to increase as the plants approach physiological maturity.

Goldenrod

The stems and roots of native goldenrods are relatively low in resin, and have such low benzene extracts that they probably contain no rubber. Leaves of *Solidago altissima* L. collected in the Ottawa district yielded from 0.1 to 1.5% of rubber. *Solidago rugosa* Mill. has a rubber content in the leaves of 0.5 to 1.3%. The highest values in both species were obtained just prior to the time of leaf-fall. Leaves from isolated collections of other goldenrods from the vicinity of Ottawa were shown by a series of 44 analyses to contain still lower amounts.

Milkweed

The developmental stage at which milkweed leaves are collected has a marked effect on the resin and rubber content. These effects are shown graphically in Fig. 1. Some of the minor variations may be attributed to effects of site, others to individual and clonal differences (6). The plotted values up to September were from analyses of oven-dried small collections. Thereafter large collections were air-dried and the material underwent a measure of fermentation during drying. The peak value of 4.6% was from yellowed leaves picked from the plants and from the ground. Maximum rubber contents occur about the time of the first frosts. Five days after the first frost about 40% of the rubber had disappeared from the leaves.

As reported for goldenrods (9), the lower leaves of milkweed have the highest rubber contents. Analyses of leaves from an early July collection

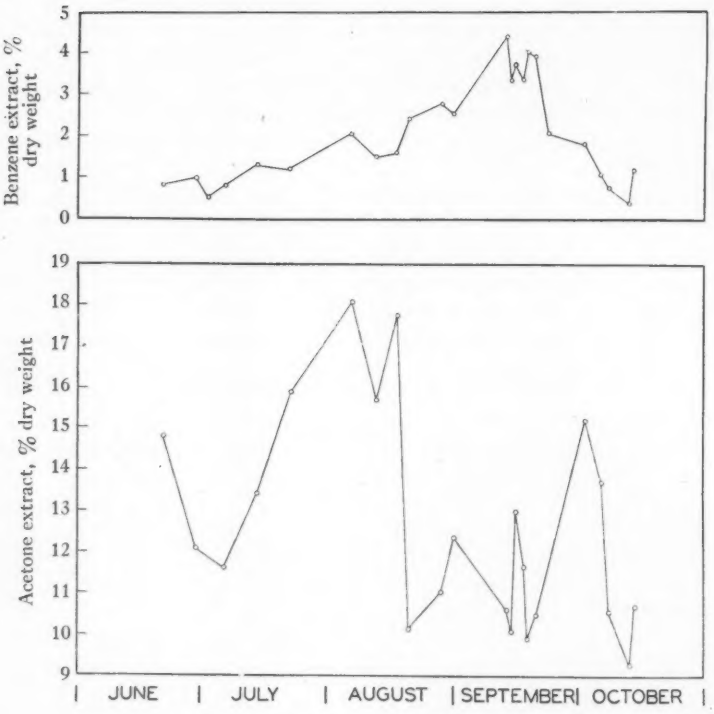

FIG. 1. *Seasonal trend of resin and rubber content in milkweed leaves.*

showed rubber contents of 0.8 to 0.9% in the leaves nearest the ground at the fourth node, with values decreasing to 0.4% in those at the twelfth node.

Milkweed stems collected in June and July, 1942, in the Ottawa district showed average resin and rubber contents of 15.9 and 0.4%, respectively. Similar analyses of June and July collections during 1943 gave corresponding average percentages of 12.4 and 0.8. A wide variety of local sites was included in the 1943 as in the 1942 collections, so that the difference is assumed to be an annual effect.

Resin–rubber contents of seed pod hulls averaged 8.8 and 0.6%, respectively. The papery placentae yielded 19.3% resin and 7.3% rubber.

Leaves and stems of *Asclepias sullivantii* collected at Walpole Island, Lambton County, Ontario, on July 20, 1943, showed resin contents of 8.7 and 6.1% and rubber contents of 1.2 and 0.2%, respectively.

Discussion

Analytical studies show that the rubber content of the common milkweed is quantitatively higher than that of any other native species investigated. Resin contents are highest in the leaves of the common dogbane. The resin–

rubber yields of the five native goldenrods examined are too low to warrant their use in studies of possible methods of mechanical extraction. The low rubber contents in Canadian members of *Solidago* species compared with the much higher amounts found in the same species in the southern parts of the range (13) suggest that the farther south goldenrods are grown the higher is their rubber content. Milkweed from southern regions of the U.S.S.R. was stated by Stepanov (16) to be richer in rubber than that from the central and northern provinces.

The findings of Kassner (7), Gerhardt (4), and Stepanov (16) that the rubber content of milkweed leaves increases with the advancing season are confirmed. Accumulation of rubber in the leaves appears to continue to the time of frost. Various factors affect the rate of increase. Yellowing of the leaves, with the consequent decrease in their dry weight, results in an apparent increase in rubber content. If yellowing takes place prematurely an increase in rubber above the average is revealed. Conspicuously yellowed leaves collected in mid-August showed 2.8% rubber compared with the average of about 2% for green leaves at this date. Two mid-September 1942 collections of mixed yellow and dead leaves picked from the plants and soil showed 4.6 and 4.1% of rubber. These percentages obviously do not represent an average for the period. The accentuated peak in Fig. 1 contains these two results along with others from large September collections in which the rubber was probably concentrated by partial retting during drying. Points on this curve show the wide variations to be expected in material from wild stands. The curve shown in Fig. 2 is believed to be approximately representative of the average rubber content of leaves from wild stands in the Ottawa district. It is intentionally stripped of the variations attributable to differences in site, and individual and clonal variations. It is also modified (see Fig. 1) in the region of maximum rubber content for abnormalities such as those mentioned above.

The rubber content of mid-September plants from wild stands in the Ottawa district approximates the yields from plants in southern Russia and the Southern United States. For example, Rheineck (15) reports that Klein investigated the native latex-bearing plants of Austria in 1917 and found *A. syriaca* to contain the most rubber, namely, about 2% in the leaves and 0.28% in the stems. Hall and Long (6) found the highest rubber content for *A. syriaca* to be 4.4% in yellow leaves collected at Lincoln, Nebraska, in late October. The rubber content in a two-year old plantation in Iowa reached 2.85% in early October (4). Stepanov (16) found the maximum rubber contents of leaves from wild stands three years old and older in Russia to be 4.4%. The best selected clones from Russian plantations (1939) gave 6.3% rubber.

Wide variations in resin content are found in wild plants throughout the growing season. From Fig. 1 it appears that a decline in resin content takes place during the period in which rubber values are at a maximum. This confirms the results of Stepanov (16). Gerhardt (4) found that the resin

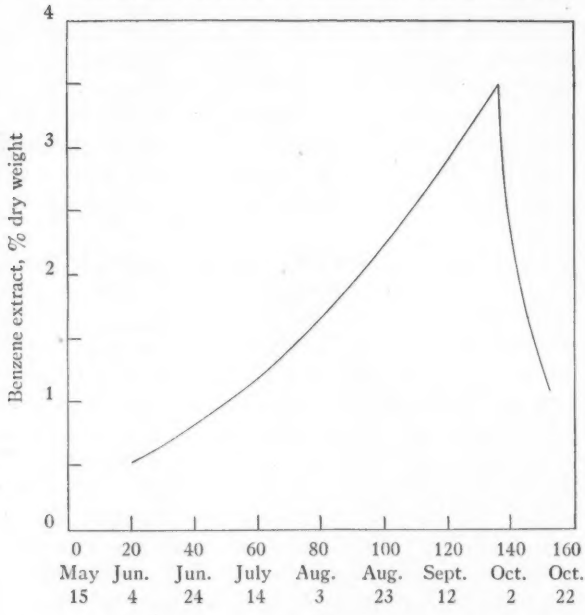

FIG. 2. *Rubber content of milkweed leaves in Ottawa district.*

content remained almost constant throughout the summer and declined slightly in September and October. Both authors obtained their data from plantation material.

The rubber content of milkweed stems averages about 0.5%, a value that makes processing stems for rubber dubious. The gross resin fraction of the stems ranges from 8 to 18% and extraction for the resin appears more practical. The percentage of rubber in the stems appears to decrease as the plants approach maturity, a correlation inverse to that found in the leaves.

Acknowledgments

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RESIN–RUBBER FROM CANADIAN GROWN PLANTS

II. METHOD OF EXTRACTION FROM THE COMMON MILKWEED, *ASCLEPIAS SYRIACA* L.[1]

By N. H. Grace[2], R. W. Watson[2] and J. Klassen[3]

Abstract

Resin–rubber gum containing about 40% resin, 35% rubber, and 25% residue insoluble in either acetone or benzene, has been extracted from milkweed leaves by a mechanical method. The extraction method involves cooking leaves in dilute alkali, washing until the pH is reduced to about 9.5, and pebble milling to effect agglomeration. Sodium hydroxide solution (1.5%) boiling at atmospheric pressure has been used with cooking periods of from 2½ to 3 hr. Gum yields of the order of 8% of the weight of dried leaves have been obtained.

Resin–rubber gums also have been obtained by this method from the stalks and seed pod hulls of milkweed. Similar materials have been extracted from a number of other plants.

Introduction

Development of simple mechanical methods for the extraction of rubber from Canadian grown plants has been the major object of this investigation. Preliminary studies of the resin–rubber content of species of three promising genera resulted in the selection of common milkweed as the native plant with the highest known rubber content (13). For this reason the common milkweed has been utilized in the following investigations.

There is almost no available literature on mechanical methods for the separation of rubber from the leaves of plants such as milkweeds, goldenrods, or dogbanes. Solvent extraction has been the method reported (7). A mechanical method (3) claimed effective for woody plants with low rubber content is not applicable to leaf tissue. Some information is available on Russian methods for the extraction of rubber from kok-saghyz roots (1, 5, 12). These methods, however, also are not applicable to milkweed or other leaf-rubber plants. The method developed for the extraction of rubber from guayule (9) appears practicable, with some modifications, in the extraction of kok-saghyz rubber, but requires drastic modification when used with milkweed.

Materials and Equipment

Methods of collecting, drying, and analysing milkweed leaves, stems, and pods have been described (13). These studies involved numerous collections of 100 to 2,000 lb. of whole fresh plants taken within five miles of Ottawa. The identity of collections was maintained because of possible effects attribut-

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[2] *Biochemist, Industrial Utilization Investigations.*

[3] *Chemical Engineer, Industrial Utilization Investigations.*

able to site or developmental stage on the quantity and quality of the resin–rubber fraction. Air-dried material was used in most of the work, though fresh material was used in some experiments.

Plant material was cooked in laboratory glassware heated by gas burners. In experiments involving charges of more than one kilogram of air-dried material, cooking was done in an iron drum, gross capacity about 120 litres, heated by a gas burner or direct steam injection. Pressure cooking took place in a laboratory autoclave.

Four sizes of pebble mills, quart, 1-, 4- and 12-gal. gross capacity were used. A gang of six quart-mills permitted simultaneous processing of six samples. Likewise, two 1-gal. mills were operated by one mechanism. The mills rotated at 80, 61, 48, and 53 r.p.m. in order of ascending volumes. The calculated 80% critical speeds were 105, 78, 61, and 53 r.p.m. Only the 12-gal. mill was provided with a variable speed drive. Unless otherwise specified, the mills were approximately half-filled with flint pebbles. Quart mills were charged with pebbles whose average diameter was from $\frac{1}{2}$ to $\frac{3}{4}$ in. Average pebble diameters for the other mills ranged from 1 up to $1\frac{3}{4}$ in.

Cooked plant material was washed on a filtration unit in which the filtering medium consisted of four to eight layers of cheesecloth suspended in hammock fashion or supported on a coarse galvanized iron wire screen.

In some experiments a vibrating 60 mesh stainless steel screen was used.

A galvanized sheet iron Soxhlet type extractor accommodating about 10 lb. of ground plant material and heated by steam or hot water was used in studies on solvent extraction.

Experimental

A number of procedures for the extraction of rubber from milkweed were investigated. The investigations were not designed to produce elaborate scientific data. Modes of extraction that indicated great difficulty or impracticability were dropped, and stress was laid on those with greater promise. Short cuts were taken wherever possible in an effort to obtain samples that could be evaluated by the rubber laboratory as to quality and possible value as blending agents.

Preliminary study of methods of extraction was paralleled by solvent extraction as a ready method of obtaining test samples of milkweed rubber. Air-dried ground leaves were subjected to successive acetone and benzene extractions (13). The benzene extract was concentrated under reduced pressure with added antioxidant (phenyl-β-naphthylamine) and precipitated and washed with either methyl alcohol or acetone. The coagulum was dried *in vacuo* and stored in the dark.

Latex has been laboriously collected drop by drop from the freshly cut milkweed stalks (6). Experimental study of latex drainage has involved placing the freshly cut ends of stalks in dilute ammonia or sodium salicylate solutions, or leaching freshly crushed whole plants.

Fermentative decomposition has been reported as a method of producing rubber rich concentrates and improving the quality of the rubber (8, 10). Anaerobic fermentation of fresh and dried milkweed leaves was studied. The laboratory experiments involved packing whole plants or leaves into closely covered glazed earthenware crocks and holding for periods of from 10 to 21 days at room temperature. The coarse fragments were separated by a screen from the bulk of finely divided material. The fine suspension was then subjected to centrifugation.

Similarly, chemical treatments can be used to produce concentrates. Both dilute alkalis and acids were utilized, usually sodium hydroxide and sulphuric acid in the concentration range of from 1 to 3%. In some experiments alternative alkali and acid treatments were applied. Leaves were cooked for periods of one or more hours in an open cooker at the boiling point.

Leaves and whole plants were subjected to crushing and grinding, and to extensive pebble milling. Separation into rubber-rich and rubber-poor fractions by settling from suspensions, screening, and slow flow and settling over trays 16 ft. long were considered.

Mechanical Separation of Resin–rubber Gum

A combination of chemical treatments and subsequent mechanical processing has been given detailed attention. This involved cooking the air-dry leaves in dilute alkali, washing, draining, and pebble milling. Leaves were boiled in 1.5% commercial sodium hydroxide solution (also acid in some experiments) for two to four hours. They were then washed until the pH was reduced to about 9.5. After drainage the water content was 90 to 92%. The pebble mill was charged with the drained material and an equal weight of water, and milled for 18 to 24 hr. While this was the milling procedure used in most of the experiments about to be described, a superior but subsequently developed method involved "thick milling," i.e., the cooked drained leaves are milled for six to eight hours, and then an equal volume of water is added. Mills were not charged in excess of 60% of the total capacity.

Effects of cooking, washing, and pebble milling on extraction of resin–rubber gums will be shown by the results of nine representative series of experiments. For the sake of convenience these are designated Series *A* to *I*, inclusive.

Cooking

Series A. Samples of milkweed leaves were cooked in dilute alkali and acid solutions over a range of concentrations in the autoclave at 120° C. for a period of two and one-half hours. Record was made of the dry weight of drained residue from 200 gm. of leaves cooked in a volume of 3.5 litres.

Series B. Duplicate 50-gm. samples of air-dried leaves were cooked for four hours in one litre of sodium hydroxide solution. Concentrations of 1.5, 3.0, 6.0, and 9.0% and quart pebble mills were used. Leaves were collected September 20, 1942. They contained 12.8 and 2.2%, respectively, of acetone- and benzene-soluble fractions.

Series C. Cooking periods of one to five hours were used with 200 gm. samples of air-dried leaves in 3 litres of 1.5% sodium hydroxide solution. Prior to alkaline treatment the leaves had been cooked twice in 3 litres of water for 30 min. Resin–rubber was extracted in 1 gal. pebble mills. Leaves showed 11.9% acetone- and 2.1% benzene-soluble fractions, respectively.

Washing

Series D. Samples of air-dried leaves weighing 200 gm. were cooked in dilute sodium hydroxide and washed to different pH values. Addition of dilute sulphuric acid after preliminary washing was used to reduce the pH to 5.5. Resin–rubber gum was extracted in 1 gal. pebble mills and the amount of resin and rubber in the gum determined.

Pebble Milling

Series E. Three hundred grams of air-dried leaves was cooked in 6 litres of 1.5% sodium hydroxide solution for three hours, washed, drained 24 hr., and then weighed. The cooked drained material was divided into six equal amounts of 148 gm. Leaves were from a late September 1942 collection, with an acetone-soluble fraction of 11.7%, coefficient of variability 6.2%, and a benzene-soluble fraction of 3.8%, coefficient of variability 5.0%. The preparation of a duplicate lot of six samples from 300 gm. resulted in drained weights of 175 gm. A mixed lot of leaves had to be used; the resin and rubber contents of this composite sample were not determined. Each group of six extractions was made in quart pebble mills. Mills were charged 25, 37, 56, and 68% full of pebbles, and, in the first part of the series, with 148 gm. of drained leaves and 113 gm. of water, and, in the second part, with 175 gm. and 50 gm. of water. The effect of pebble charge on agglomeration was determined (charges of 261 and 225 gm., respectively, for the different leaves provided approximately similar viscosity and milling behaviour). Effects of slurry consistency, in conjunction with a 56% pebble charge, were determined by milling at the above consistencies, and also with both increase and decrease of water content by about 20%.

Series F. Two 1-kgm. and one 900-gm. samples of the September 20 collection, Series B, were cooked for five hours in 1.5% sodium hydroxide solution, washed, and drained. Water was added to give charges of 4800, 3300, and 3000 gm., respectively, and the 4-gal. pebble mill was used for agglomerating resin–rubber.

Series G. Three and one-half kilograms of air-dried leaves was cooked one hour in water, drained, and then cooked for three hours in 50 litres of 1% sodium hydroxide solution. Acetone- and benzene-soluble fractions of the leaves were 10.9 and 4.9%, respectively. Resin–rubber was extracted in the 12-gal. mill.

Effects of Various Chemical Treatments

Series H. Samples of 50 gm. of air-dried leaves of the collection described under Series B were subjected to a number of different chemical treatments and resin–rubber was agglomerated in quart pebble mills. Treatments included

sodium hydroxide alone, followed by acid, and, in some experiments, further alkali. In some of the experiments ammonium hydroxide, calcium hydroxide, and sodium silicate were used. Variations in sequence of treatments was accompanied by a range of cooking times. In all experiments one litre of solution was used. Four of these extractions involved duplication but these duplicates were agglomerated in pebble mills provided with a rubber covered lid. This condition permitted investigation of agglomeration on a rubber surface.

Extraction of Resin–rubber from Midsummer Collections

Series I. Collections of leaves taken during July and August were extracted in the 12-gal. pebble mill. Samples of 2500 gm. of air-dried material were cooked in 37.5 to 40 litres of 1.5 to 2.5% sodium hydroxide solution for four hours. The object of these experiments was to determine the nature of the extraction from immature leaves, the type of coagulum, and relative amounts of resin and rubber. The work involved was expected to provide information helpful in the initiation of operations on a pilot plant scale.

Analysis of Resin–rubber Gums

The resin and rubber contents of concentrates and gums have been obtained by modifications in the procedure developed for leaf tissue (13). Two to three grams of dried gum is spread thinly on a tared Whatman 50 filter paper, placed in a cellulose thimble capped with cotton wool, and extracted for 24 hr. with acetone in a Soxhlet. The thimble is air-dried for eight hours at 80° C. and further extracted for 24 hr. with benzene. Hereafter the terms "resin" and "rubber" are used when reference is made to acetone- and to benzene-soluble extracts.

The rubber hydrocarbon obtained from solvent extraction of leaves or resin–rubber gum has been determined by the chromic acid oxidation method and also by iodine absorption (2, 4).

Extraction of Resin–rubber from Milkweed Stalks and Seed Pod Hulls, and from Other Plants

Resin–rubber gums have been extracted from milkweed stalks and seed pod hulls by the methods described. Similarly, preliminary extractions of gums have been made from spreading dogbane (*Apocynum androsaemifolium* L.), the common goat's-beard (*Tragopogon pratensis* L.), sow thistle (*Sonchus* spp.), and a wild lettuce (*Lactuca* sp.). Use of flotation as an aid in extraction of these species has been reported (11).

Results

The percentages of resin and rubber obtained by successive large-scale acetone and benzene extractions were approximately equal to those reported from laboratory analyses (13). The quality of the rubber varied markedly with plant development. Late August or September collections of leaves yielded an oily rubber fraction suggesting existence in the leaf of a low polymer (or partial depolymerization during extraction). In contrast, extracts from

July or early August collections were much less sticky and considerably more elastic. A typical late July collection yielded hydrocarbon with iodine unsaturation of 83.1% and a chromic acid value of 85.3.

Great variation in quantity, quality, and practicability were shown by the extraction methods investigated. Latex drainage from cut ends of plants provided small quantities for research purposes (6), but it is applicable only on a laboratory scale. Fermentative decomposition of fresh tissue produces resin–rubber concentrates. One 10-day fermentation provided a concentrate that was separated by centrifugation. This contained 31% resin and 42% rubber. Dried tissue ferments much more slowly. While this method appears practical it presents a number of difficulties. Resin–rubber also may be concentrated by acid and alkaline treatments. Cooked material containing 16.7 and 8.7%, respectively, of resin and rubber was obtained from leaves subjected to the usual 1.5% sodium hydroxide solution. Respective initial resin and rubber contents were 13.0 and 3.8%. Cooking reduced the original weight to approximately one-third. While alkaline treatments frequently produced a fine floating scum of resin–rubber, the small amount thus produced renders the method impractical. Grinding and crushing treatments alone failed to produce appreciable quantities of resin–rubber from fresh or dried tissue.

Mechanical Separation of Milkweed Resin–Rubber

The results of the various series of experiments as they bear on cooking, washing, and pebble-milling, follow. Subsequently, data are given for effects of special treatments and varying types of material.

Cooking

Results of Series *A* are graphically shown in Fig. 1. It is apparent that alkali is somewhat more effective than acid in dissolving out leaf material. When less than 1% sodium hydroxide is used there is difficulty in the subsequent agglomeration in the pebble mill. Consequently the concentration of 1.5% was selected. Approximately one-half of the alkali at this concentration is combined. Less alkali may be used if the leaves have been previously water-extracted. Effects obtained by the use of still higher alkali concentrations are shown by the results of Series *B* in Table I. The yield of gum decreases when the concentration of sodium hydroxide is raised above 1.5%, but the relative percentages of resin and rubber do not vary appreciably. Results for duplicate samples indicate the order of agreement obtained in such extractions.

The yield of gum is correlated with the duration of the cook as shown by results for Series *C* graphically presented in Fig. 2. It is apparent that optimum yields of dry gum follow cooking in an open vessel for a period of between 2½ and 3½ hr. The resin of the gums varied between 35 and 37% and the rubber between 29 and 31%. Time of cooking did not change the grade of gum appreciably. The period may be reduced by using pressures

higher than atmospheric, and this technique reduces fragmentation of the leaves and so results in more rapid filtering and washing.

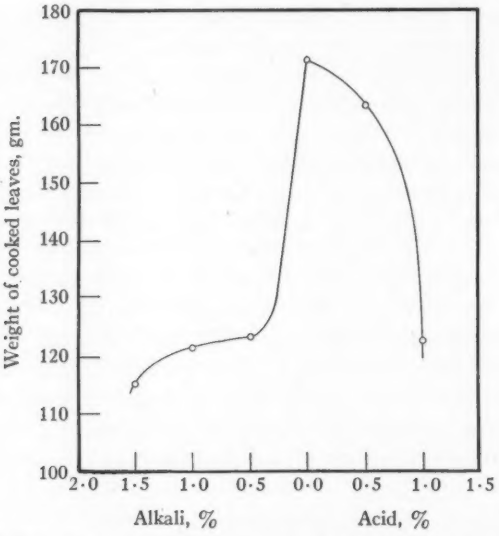

FIG. 1. *Effects of dilute sodium hydroxide and sulphuric acid solutions on dry weights resulting from cooking of 200 gm. milkweed leaves for 2½ hr. in the autoclave at 120° C.*

TABLE I

EFFECTS OF ALKALI CONCENTRATION ON EXTRACTION OF RESIN–RUBBER FROM MILKWEED LEAVES IN QUART PEBBLE MILLS

(Leaves from plants collected in one vicinity, September 20, 1942). Series B

Concentration of sodium hydroxide, %	Analysis of resin–rubber gum			
	Wet weight of gum, gm.	Resin, %	Rubber, %	Detritus by difference, %
1.5	5.5 5.5	37.3 46.5*	31.2 38.6	31.5 14.9
3.0	4.4 4.3	39.4 39.7	39.0 39.8	21.6 20.5
6.0	3.1 3.0	34.6 37.5	30.6 38.0	34.8 24.5
9.0	3.1 3.0	38.4 35.5	32.2 36.5	29.4 28.0

* *Values for duplicate extractions offset.*

Fifty grams of air-dry leaves cooked in one litre specified sodium hydroxide solution for four hours.

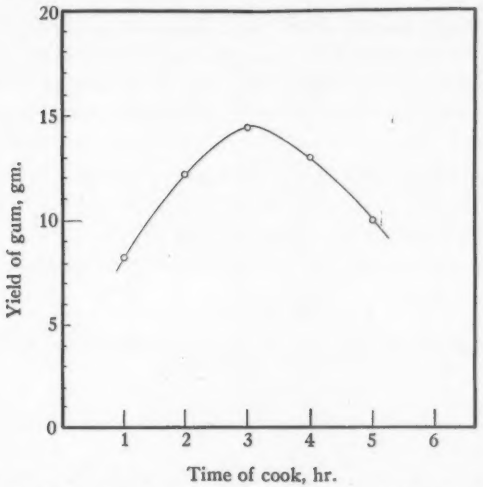

FIG. 2. *Effects of duration of cooking in 1.5% sodium hydroxide on yield of gum from 200 gm. of milkweed leaves.*

Washing

It is necessary to remove the alkaline liquor after cooking and reduce the pH substantially if effective gum agglomeration is to be obtained. Results of Series *D* are shown in Fig. 3, where weight of resin and rubber extract

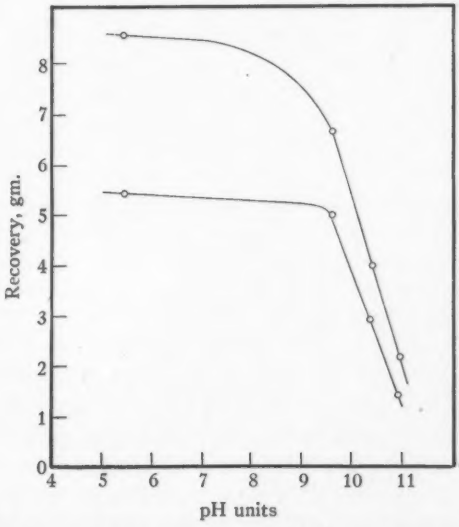

FIG. 3. *Recovery of resin (acetone extract) and rubber (benzene extract) from gums obtained from 200 gm. of milkweed leaves after cooking in dilute sodium hydroxide and washing to different pH values. Upper curve—acetone extract; lower curve—benzene extract.*

(determined analytically on the dried crude gum) are plotted against the pH to which the leaves were washed. It is apparent that a sharp decline in the recovery of rubber and resin occurs if the pH of the leaves is 10 or higher. The inflexion in the curve is not quite as sharp for the acetone extract.

Pebble Milling

Effects of pebble charge and slurry consistency are described by the data of Table II from results of Series *E*. Maximum gum yield occurred at pebble charges of 37 and 56% for the two different lots of leaves. The data are too meagre to be other than suggestive in regard to these maxima. Analytical

TABLE II

EFFECTS OF PEBBLE CHARGE AND SLURRY CONSISTENCY ON EXTRACTION OF RESIN–RUBBER IN QUART PEBBLE MILLS

Series E

Pebble charge, % of mill capacity	Slurry consistency		Yield of dry gum, gm.	Gum analyses			Recovery calculated on dry leaf weight	
	Drained* leaves, gm.	Water added, gm.		Resin, %	Rubber, %	Detritus, %	Resin, %	Rubber, %
68	148†	113	3.88	34.1	32.4	33.5	2.81	2.68
56	148	113	3.19	38.1	37.4	24.5	2.60	2.53
37	148	113	5.05	35.7	38.3	26.0	3.83	4.11
25	148	113	4.29	35.9	38.2	25.9	3.28	3.49
68	175	50	4.34	38.8	34.4	26.8	3.65	3.24
56	175	50	5.17	39.4	32.1	28.5	4.43	3.61
37	175	50	4.23	45.4	33.8	20.8	4.17	3.11
25	175	50	4.49	43.0	33.2	23.8	4.20	3.24
56	148	165	3.17	39.4	38.5	22.1	2.66	2.60
56	148	113	3.19	38.1	37.4	24.5	2.60	2.53
56	148	67	1.72	36.0	17.6	46.4	1.32	0.64
56	175	95	4.30	42.9	31.4	25.7	4.00	2.93
56	175	50	5.17	39.4	32.1	28.5	4.43	3.61
56	175	5	0	—	—	—	—	—

* *Each lot of cooked drained leaves came from 50 gm. of air-dried material.*

† *Drained weights of 148 gm. represent leaves with average acetone- and benzene-soluble extracts of 11.74 and 3.83%, respectively.*

values indicate that pebble charge in the range covered has no effect on resin and rubber content of the coagulum. Effects attributable to slurry consistency are clear-cut. When the pebble charge is fixed at 56%, increase in the water content of the slurry by about 20% has little effect on either yield or gum analyses. Conversely, however, reduction in water content greatly reduced gum recovery for one collection of leaves and also led to a much lower rubber hydrocarbon content in the gum. The second sample failed to yield any gum under these conditions. A feature of the results is the high recovery

of rubber fraction. In some of the better extractions, recovery approached 100% of the rubber in original leaves. Further, effects of slurry consistency on gum agglomeration in a 4-gal. pebble mill are described in Table III, which presents results from experiments of Series *F*. Optimum agglomeration in this particular pebble mill required the use of a much thicker slurry than with the quart mills. Reduction in total charge from 4800 to 3300 gm., for leaves weighing 1000 gm. before cooking, resulted in a very great increase in gum recovered. However, the coagulum from the thicker slurry contains, as might be expected, substantially more detritus.

TABLE III

EFFECTS OF SLURRY CONSISTENCY ON AGGLOMERATION IN THE 4-GAL. PEBBLE MILL*

Series F

Weight of air-dry leaves, gm.	Weight of slurry in mill, gm.	Resin–rubber gum			
		Wet weight, gm.	Resin, %	Rubber, %	Detritus (by difference), %
1000	4800	15.5	44.2	35.1	20.7
1000	3300	105.0	33.3	29.7	37.0
900	3000	125.0	32.4	25.3	42.3

* *Leaves cooked in 16 litres of 1.5% sodium hydroxide for five hours.*

Extent of recovery on the largest-scale milling used in the laboratory may be noted from the results of Series *G*. The washed, cooked leaves contained 17.2 and 6.7% resin and rubber, respectively. After 19 hr. milling, the dried slurry contained 2.3% resin and 0.3% rubber, indicating that about 95% of the rubber in the slurry had been extracted. Similarly the recovery of resin, based on the content of slurry at the start of milling, was of the order of 87%. The washed wet gum weighed 510 gm., had a water content of 23% and resin- and rubber-contents of 37 and 29%, respectively. Consequently 145 gm. of resin and 114 gm. of rubber were obtained in the agglomerate. Correcting the original dry weight of leaves for 10% moisture content, it may be calculated that the rubber extracted amounted to 74% of that present in the leaves. Similarly about 42% of the resin was recovered. It is obvious that the loss of rubber occurs during the washing rather than the milling operation. Such losses in washing have been encountered during the processing of batches substantially larger than the 50 to 200 gm. readily handled in the laboratory.

Effects of Various Chemical Treatments

In Table IV are given data for the results of the experiments of Series *H* in which agglomeration followed various chemical treatments. When sulphuric acid is used for one-half hour, after sodium hydroxide of the same concentration, yield and resin content are increased, rubber content is not affected, and

TABLE IV

EFFECTS OF VARIOUS COOKING PROCEDURES ON RESIN–RUBBER EXTRACTION FROM MILKWEED LEAVES IN QUART MILLS

Series H

Cook			Resin–rubber gum			
Chemical	Conc. of solution, %	Time, hr.	Wet weight, gm.	Resin, %	Rubber, %	Detritus (by difference), %
NaOH	1.0	3	4.0	36.2	25.6	38.2
			5.0†	33.6	25.7	40.7
NaOH	1.0	3	6.0	54.2	24.7	21.1
H_2SO_4	1.0	½	6.5	53.6	24.1	22.3
NaOH	1.0	3	5.9	45.9	32.3	21.8
H_2SO_4	1.0	½	4.6	40.3	34.1	17.6
NH_4OH	1.0	¼				
NaOH	1.0	3	3.6	44.9	36.5	18.6
H_2SO_4	1.0	½	4.3	44.0	35.8	20.2
Na_2SiO_3	1.0	¼				
NaOH	3.0	1				
H_2SO_4	2.0	2	3.9*	54.4	40.9	4.7
NaOH	3.0	1				
Na_2SiO_3	1.5	2				
H_2SO_4	1.5	1	2.5	47.6	38.9	13.5
NaOH	3.0	1				
Na_2SiO_3	1.5	2				
H_2SO_4	1.5	1	5.3	44.5	35.2	20.3
NaOH	1.5					
$Ca(OH)_2$	3.0	2				
H_2SO_4	1.5	1	4.2	40.3	34.8	24.9
NaOH	3.0	1				
NaOH	1.5	2				
H_2SO_4	1.5	1	2.6	44.7	46.1	9.2
NaOH	3.0	1				
NaOH	1.5	2				
H_2SO_4	1.5	1	4.7	47.4	42.7	9.9
NaOH	1.5	1				

** Actually 200 gm. of leaves was milled in a 1-gal. mill but result is reported on a 50-gm. basis.*

† Offset values for identical treatments but agglomerated in mill with rubber-covered end.

detritus greatly reduced. Alternate alkali-acid-alkali treatments produced the same general results with the exception that the rubber content was substantially increased. Some of the more drastic treatments reduced gum yield. Sodium hydroxide is a more effective cooking agent than sodium silicate or calcium hydroxide. Gums with a detritus content of less than 10% had a density of less than 1; the usual gum has a density of about 1.1.

The offset data for the first four treatments of Table IV indicate that a rubber-covered end in the pebble mill has no effect on either yield or grade of gum.

Extraction of Resin–rubber from Midsummer Collections

In Table V are given data for the extractions of Series *I*. The average resin and rubber contents of the gums were respectively about 40 and 22%. It is apparent that these gums contain less rubber than is obtained from mature leaves. Increase in cooking period in one instance suggests a decrease in gum yield. Variation in alkali content or period of collection had no clear effect on content of resin or rubber.

TABLE V

EXTRACTION OF RESIN–RUBBER FROM 2.5 KGM. MILKWEED LEAVES IN A 12-GAL. PEBBLE MILL

Series I

Date of collection, 1943	Cooking conditions		Dry weight of gum, gm.	Gum analyses		
	NaOH, gm.	Volume of solution, litres		Resin, %	Rubber, %	Detritus (by difference), %
July 14	665	37.5	89	42.5	24.7	32.8
July 21	1000	40	156	44.5	22.8	32.7
July 28	850	40	174	41.5	19.9	38.6
August 3	800	40	217	34.6	21.9	43.5
August 10	650	40*	117	44.8	22.9	32.3
August 10	650	40	213	44.6	22.2	33.2
August 14	750	37.5	127	41.7	23.2	35.1
August 14	650	40	138	41.9	18.2	39.9

**Cooking period, seven hours; all others, four hours.*

It was determined that midsummer collections of leaves could be processed by the laboratory methods developed for mature tissue. Among the difficulties encountered was a tendency for the formation of small hard flakes of coagulum which failed to agglomerate further. These flakes could be separated readily from the slurry by the vibrating screen and further agglomerated by a short period of pebble milling in fresh water.

Resin–rubber Gums

Gums from leaves are dark in colour and tend to be quite tacky and have a density of about 1.15. Resin–rubber gums from stalks and seed pod hulls are light in colour, usually less tacky, and have about the same density. These gums are substantially richer in resin and poorer in hydrocarbon fractions. Gums from the other species mentioned also were relatively high in resin and low in hydrocarbon.

Typical gums from mature leaves of a 1942 collection and seed pod hulls of the same year yielded resin and hydrocarbon fractions following extended solvent extraction. The analysis of the leaf gum yielded 32.5% resin and

31.1% rubber. Corresponding values for pod gum were 48.9 and 25.2%, respectively. Chromic acid oxidation values for the hydrocarbons were respectively from leaf and pod 87.4 and 79.4. In other determinations this value for hydrocarbon from leaf gums has ranged between 70 and 74, usually less than for hydrocarbon extracted by solvent directly from leaves.

It should be mentioned that 24-hr. acetone extraction in gum analysis does not remove all the resin fraction. Gum analyses are receiving further study.

Recommended Procedure

Milkweed leaves, stems, or seed pod hulls are cooked in dilute alkali, usually 1 to 1.5% sodium hydroxide solution, for from 2½ to 3 hr. The cooked leaves are drained and washed until the pH is reduced to about 9.5. Washed, drained material, having water content of from 90 to 92% is placed in a pebble mill and ground until resin–rubber worms appear. An approximately equal volume of water then is added and milling continued to agglomerate resin–rubber gum.

Preliminary results indicate that this extraction method may make available for study the resin–rubber fraction from a number of plants.

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